Proceedings
of the
Society
for
Experimental Biology and Medicine

Vol. 46. April, 1941. No. 4.

SECTION MEETINGS

CLEVELAND	
Western Reserve University	March 14, 1941
DISTRICT OF COLUMBIA	
George Washington University	April 3, 1941
ILLINOIS	
University of Chicago	March 4, 1941
MINNESOTA	
University of Minnesota	March 19, 1941
WISCONSIN	
Marquette University	April 4, 1941

12049 P

Sulfanilamide, Sulfapyridine, and Sulfathiazol Therapy of Gonococcal Infection of the Chorio-Allantoic Membrane.

Frederik B. Bang and Betsy Bang. (Introduced by P. H. Long.)
From the U. S. Marine Hospital, Baltimore, Md.

Until lately no satisfactory experimental animal has been available for testing the effect of drugs on gonococcal infection. The chorio-allantoic membrane of embryo chicks has recently been infected with gonococci and treated successfully with sulfanilamide.[1, 2]

We present here a comparison of the effects of sulfanilamide, sodium sulfapyridine, and sodium sulfathiazol on such infections. We have also tested two sulfanilamide-resistant strains of gonococci for their resistance *in vivo* to these 3 drugs.

Experimental Procedure. Two strains of gonococci which had

---

[1] Marran, G., and Berry, G. P., *J. Bact.*, 1938, **36**, 280.

[2] Hill, J., and Pitts, A., *J. Urol.*, 1939, **41**, 81.

been made resistant *in vitro* to 80 mg of sulfanilamide were furnished by Dr. Carpenter[3] and were compared with 4 strains isolated from patients who did not respond immediately to drug therapy.

Using the technic of Goodpasture and Buddingh,[4] the chorio-allantoic membrane of chicks 9 to 12 days old was inoculated with one drop of a fresh saline suspension of gonococci from a recently incubated blood plate. The standard suspension contained about 100,000 diplococci per cc, and the ensuing eggs were inoculated with one drop of a suspension of similar turbidity. The eggs were incubated at 36°C for about 12 hours. Discarding dead eggs, varying amounts (see tables) of 1/100 solution of sulfanilamide, sodium sulfapyridine, and sodium sulfathiazol in normal saline were dropped on the chorio-allantoic membranes. The sodium salts of the latter two were used because of the increased solubility of these compounds. That death of the gonococci was not due to temporary alkalinity of the membrane is shown by the facts that gonococci were demonstrable 6 hours after the addition of drug, and that the sulfanilamide-resistant strains survived.

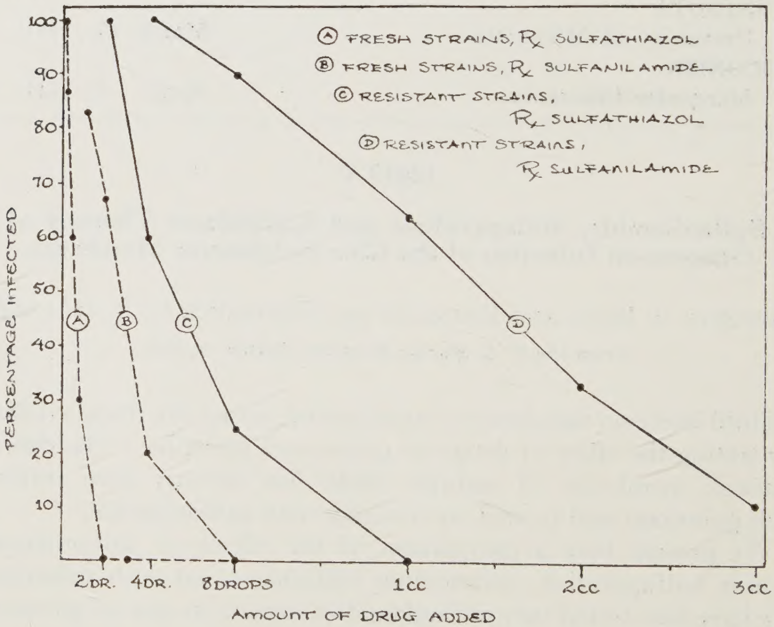


FIG. 1.

The Effect of Sulfathiazol and Sulfanilamide on Freshly Isolated and Sulfanilamide-Fast Strains of Gonococci.

---

[3] Westphal, Charles, and Carpenter, *J. Bact.*, 1940, **32**, 47.

[4] Goodpasture, E. W., and Buddingh, G. J., *Am. J. Hyg.*, 1935, **24**, 319.

After 2 days, smears and cultures of the membrane and allantoic fluid were made to determine the presence of gonococci. If either of these were positive, the egg was considered infected; if both were negative the egg was adjudged cured. Some of the apparently cured (negative) eggs had died during the 2 days, but these were included in the results since no greater percentage of dead eggs was found in any one group. Controls were used in each experiment, and only one of 30 controls failed to show infection. Histological sections of some of these controls showed that the infection did not penetrate the embryo.

Concentration of Drugs. Colorimetric tests for the presence of the drugs showed that they were distributed throughout the egg, that they were only slightly acetylated.* However, these analyses, taken at the end of the first and second days, accounted for less than half of the drug added. This may indicate that the embryo is able to metabolize the drug.

Results. As shown by Tables I and II, the sulfanilamide-resistant strains grew at much greater concentrations of all 3 drugs than did the recently isolated strains.

TABLE I.
Freshly Isolated Strains of Gonococci.

Drug added Drops to 1-100 sol.	Sulfanilamide		Sulfapyridine		Sulfathiazol	
	Treated	Cured	Treated	Cured	Treated	Cured
⅛ dr					5	0
¼ ”					10	3
½ ”			2	0	11	5
1 ”	2	1	3	1	13	7
2 ”	2	1	2	1	3	3
3 ”	3	1	3	0	3	3
4 ”	2	1				
8 ”	3	3	2	2	4	4

TABLE II.
Sulfanilamide-fast.

Drug added Drops to 1-100 sol.	Sulfanilamide		Sulfapyridine		Sulfathiazol	
	Treated	Cured	Treated	Cured	Treated	Cured
2 dr					1	0
4 ”	3	0	2	0	3	2
8 ”	3	1	6	2	6	4
1 cc	7	3	2	1	4	4
2 ”	4	2	3	0		
3 ”	3	2	1	0		

---

* Samples of blood, embryonic fluid, allantoic fluid, amniotic fluid and yolk were analyzed by Miss Dorthea Babbitt of the Department of Pharmacology, Johns Hopkins University.

Sulfathiazol is shown to be more effective than either sulfanilamide or sulfapyridine in treating the resistant strains. This agrees with the clinical reports of the efficacy of sulfathiazol.[5, 6]

Statistical Method. Curves were plotted by accumulating negative and positive results.[7] Curves of sulfapyridine were not included because they closely paralleled those of sulfanilamide.

Summary. Sulfanilamide-resistant and freshly isolated strains of gonococci were tested for their response to sulfanilamide, sodium sulfapyridine, and sodium sulfathiazol on the chorio-allantoic membrane of embryo chicks. The resistant strains retained their resistance *in vitro.* Sulfanilamide and sulfapyridine were about equally efficient in curing the infection. Sulfathiazol was about 3 times more effective therapeutically than sulfanilamide or sulfapyridine.

12050 P

The Number of Vitamins Required by the Rat.*

L. R. RICHARDSON, A. G. HOGAN, BARBARA LONG AND K. I. ITSCHNER.

From the Laboratory of Agricultural Chemistry, University of Missouri, Columbia, Mo.

During their studies of rat dermatitis Richardson and Hogan[1] observed that rats which receive only the recognized water-soluble vitamins are able to survive for long periods of time, and to maintain a moderate rate of growth. Four of the 8 females described in Groups III and IV, Table I, of the report cited, attained an average weight of 176 g in 20 weeks, when it became necessary to discard them. The ration was then changed in various ways in an attempt to secure a more rapid growth rate and 3 of the more important modifications are described in Table I. The response of the animals is shown in Fig. 1.

---

[5] Mahoney, J. F., Wolcott, R. R., and Van Slyke, C. J., *Am. J. Syph., Gon., and Venereal Dis.*, 1940, 613.

[6] Burkholder, T. M., and Bang, F., *J. Urol.*, 1940, **44**, 541.

[7] Reed, L. J., *Biological Effects of Radiation*, 1936, VI, 227.

* Contribution from the Department of Agricultural Chemistry, Missouri Agricultural Experiment Station, Journal Series No. 680.

[1] Richardson, L. R., and Hogan, A. G., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 583.

TABLE I.
Description of Rations

Constituent	Ration A	Ration B	Ration C
Casein	20	20	20
Sucrose	71	73	65
Lard			8
Salts	4	4	4
Cellulose	3	3	3
Cod liver oil	2	0	0
		Daily allowance per rat	
Linoleic acid*		10 mg	
Concentrate of vitamins A and D			3 mg daily per rat
			Mg per 100 g ration
Carotene		50 μg	
Calciferol†		0.125 ”	
Alpha-tocopherol‡		25 ”	2.5
Thiamin‡	0.2 mg§	20 ”	0.8
Riboflavin‡	0.4 ”	20 ”	1.6
Vitamin B_6‡	20 μg‖	20 ”	1.2
Ca-pantothenate‡	70 ”	100 ”	1.0
Choline‡		10 mg	400

*Generously supplied by Dr. J. B. Brown of Ohio State University.
†Generously supplied by the Winthrop Chemical Co., Inc., New York.
‡Generously supplied by Merck and Co., Rahway, N. J.
§Per 100 g ration.
‖Daily allowance.

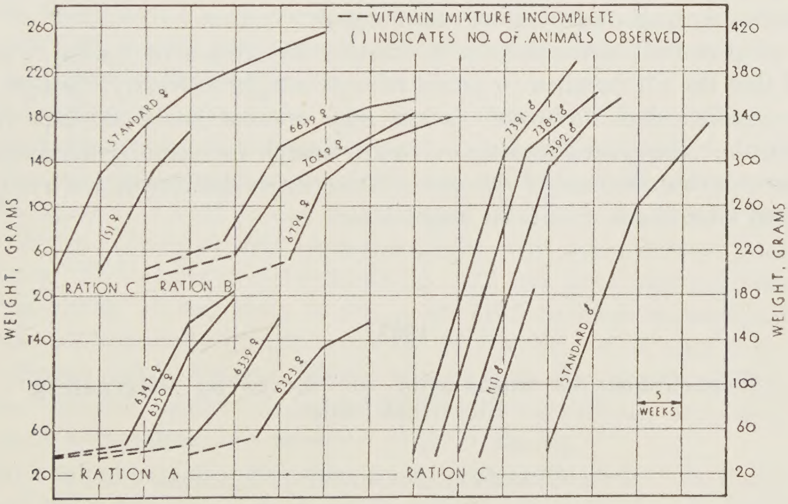


FIG. 1.

The rats which were changed to Rations A and B recovered from dermatitis,[1] and were retained to show whether recovery is permanent. The surprisingly rapid gains in weight suggested the use of Ration C, in order to improve the rate of growth. Of the older rats, Nos. 6639, 7049, 7385, 7391, 7392 are still alive and thrifty. The others were healthy when discontinued. The standard growth curves were plotted from records of the stock colony.

It will be observed that Ration B contains no added fat, but it was supplemented with 10 mg of linoleic acid daily per rat. Ration A contains no added fat except the 2% of cod liver oil. Since neither ration supplies the quantity of fatty acids that is necessary for a normal rate of growth, even if the vitamin supply is adequate, a ration was prepared which contains additional fat. Ration C contains 8% of lard, also a more generous allowance of the water-soluble vitamins. In all 14 male and 5 female rats have received this ration and up to the present their growth rate is about the same as that of rats in the stock colony. Three males, Nos. 7385, 7391, 7392, Fig. 1, attained an average weight of 368 g in 18 weeks. They are in excellent condition and heavier than stock colony males of the same age. Each of them has sired 2 normal litters. Jukes[2] reported that a ration which contains no water-soluble vitamins, other than those now recognized, will permit female rats to attain maturity and bear litters. However, his animals grew at a subnormal rate and reared only a small proportion of their young.

The minimum amounts that will maintain normal growth have not been determined, but it is our experience that when the water-soluble vitamins are supplied at the levels commonly employed the growth rate is suboptimal. When choline was omitted from Ration C the growth rate was severely depressed. When omitted from Ration A the growth rate was unchanged.

At present it seems that the 8 vitamins included with Ration C are all that the rat requires to attain normal weight maturity, though it is possible that the basal ration may conceal some unsuspected vitamin. However that may be, in any search for unrecognized vitamins an examination of the quantitative interrelations of the recognized vitamins is of crucial importance.

12051

Experiments on Stimulation of Nerves by Alternating Electrical Fields.

J. A. GENGERELLI AND N. J. HOLTER.
From the University of California, Los Angeles.

It is customary to consider the nerve axon as a cylinder with a porous surface through which pass ions of positive sign. The ions of the opposite sign remain on the inner side of the surface and this process of diffusion is said to continue until equilibrium is reached. The result is a cylindrical double layer of charges separated by a semipermeable membrane. Stimulation is viewed as a breakdown of this equilibrium state with a resultant shuffling of the charges at the point where the stimulus is applied. This state of ionic chaos moves down the axone as a zone of depolarization and constitutes the nerve impulse. Nerves may be stimulated by mechanical pressure, by heat, by various acids, and by electric current.

---

[2] Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 625.

The setup used involved a pair of condenser plates 9½ x 4 inches separated by a distance of 7 inches. A potential difference of approximately 10,000 volts was established between these plates at a frequency of 60 cycles. A preparation consisting of the sciatic nerve and the gastrocnemius muscle of the frog was employed. The preparation was extended on a carefully cleaned glass slide and placed between the plates of the condenser.

Under these conditions it was possible to rotate the glass slide carrying the preparation to form any desired angle with the electrical lines of force between the condenser plates. Beginning at 90 degrees, the slide was rotated alternately to one side and the other of this line by increments of 5 degrees. The results indicated that the effectiveness of stimulation varied as some direct function of the longitudinal component along the nerve axis. Beginning at 70 and 110 degrees, the intensity of contraction increased until at the maximum it became tetanic.

The following experiment was then performed: The glass slide bearing the preparation was returned to its original position of 90 degrees with respect to the field. A segment of the distal end of the nerve was bent so that it formed a 90 degree angle with the rest of the nerve and thus became parallel with the electrical lines of force. No results were forthcoming until this distal segment was approximately 18 mm long; at this point faint twitches appeared in the muscle, especially on the break of the current; at 30 mm there was clonus; and at between 35 and 40 mm there was tetany. These values differed perceptibly with different preparations, and with the same preparation as a function of its freshness.

The analogue of this experiment was next performed. The distal end of the nerve was left at right angles to the electrical lines of force but the proximal end, or that nearest the muscle, was made parallel to the field. When this was done, it was found that as little as 5 mm of the proximal part of the nerve was needed for the production of pronounced tetany in the muscle.

When the sciatic nerve was cut at its point of entrance into the muscle tissue of the gastrocnemius, no results could be secured by placing the muscle preparation in the field. The result was uniformly negative regardless of the orientation of the muscle in the field. Similar negative results were obtained when the region of the nerve immediately proximal to the muscle was painted with ethyl alcohol. These experiments serve to demonstrate that the results described are associated with the phenomenon of nerve stimulation and conduction and are not due to artifactual stimulation of the muscle itself.

An experiment bearing upon the possible artifactual stimulation of the nerve by the glass slide is the following. The preparation was placed in a narrow rectangular glass container, and the system rotated in such wise that the long axis of the nerve was parallel to the lines of force of the field. When the potential difference was applied to the condenser plates, the muscle went into a state of tetany. With the field on, saline solution was poured into the container. As soon as the solution covered the bottom of the vessel the tetany instantly disappeared and the muscle became completely flaccid.

The above result may be interpreted as meaning that the electrolyte surrounding the nerve acted as an electrostatic shield and prevented the electrical lines of force from disturbing the electrical equilibrium of the nerve. This fact serves also to account for our inability to induce convulsive symptoms in white rats placed in an electrical field of 7000 volts per inch gradient. Possibly the fluids of the body were acting as an effective electrostatic shield.

These findings are in harmony with the additional fact that the reaction of the muscle was more marked when the preparation was somewhat "dried out." Immediately after dipping it in saline solution, only short twitches on the make and break of the circuit were usually obtainable under conditions which gave definite tetany when the nerve was relatively dehydrated.

12052

Routes of Administration of Materials Capable of Acting as Vitamin K.

Edwin J. deBeer, Leon Drektar and Bertrand Flusser.
(Introduced by L. Reiner.)

From the Burroughs Wellcome & Co. U. S. A. Experimental Research Laboratories, Tuckahoe, N. Y., and the Laboratory of Industrial Hygiene, New York City.

Present clinical and laboratory evidence indicates that vitamin K and many of the slightly water-soluble substances capable of acting as vitamin K are poorly absorbed from the intestinal tract under certain pathologic conditions such as ulcerative colitis, obstructive jaundice and other conditions tending to modify absorption or to restrict the production of bile salts. In the case of such interferences with the oral route, oil solutions have been injected intramuscularly. The percutaneous route, which has recently been found to be successful with such substances as stilbestrol still remained to be tested. The results of such a test are given in this paper.

Method. It was found that 2-methyl-1,4-naphthoquinone could be dissolved in warm anhydrous lanolin, liquid petrolatum, propylene glycol monostearate or solvents of similar nature. Two preparations were made, one employing anhydrous lanolin as the solvent, the other using propylene glycol monostearate. A series of graded doses, each contained in 0.25 g of material, were rubbed with the fingers into the skin beneath the wings of chicks which had previously been maintained on the Ansbacher[1] diet until clotting time, as measured by the Ansbacher[2] technic, was greater than 60 minutes. In order to insure thorough application, the rubbing was continued for a period of 5 minutes. As a further precaution, a small jacket was placed on each chick in order to prevent the possibility of the animal ingesting any vitamin K which might have escaped notice. During the application a certain undetermined portion of the material was absorbed by the ungloved fingers of the operator so that each animal actually received somewhat less than the specified dose.

Results. It was found that 2-methyl-1,4-naphthoquinone may be readily administered percutaneously. As little as 1 γ per chick reduced the clotting times of 11 out of 24 chicks to 10 minutes or less, 24 hours after dosing.

---

[1] Ansbacher, S., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 248.

[2] Ansbacher, S., *J. Nutrition,* 1939, **17**, 303.

The nature of the solvent caused no discernible difference in results.

Additional data presented in Table I show that when the dose was increased, the percentage of animals cured increased, the relative clotting times decreased and that more than 5 hours were required to reach maximum effectiveness. The greatest percentage of cures was found at the 24-hour observation period. Although the peak of activity apparently is closer to the 24-hour period than either the

TABLE I.
Venous Blood Clotting Times in Minutes Following Administration of 2-Methyl-1,4-naphthoquinone.

Mode of adminis-tration	Dose per chick, γ	Hr after injection	Clotting time in min.							Cured %
Oral	2	5	2	3	2	3	2	2		100
		24	0.8	0.5	0.5	2	0.8	0.5		100
		72	2	2.5	1.8	4.5	6	0.5		100
		144	4	>60	>60	>60	>60	6		33
Intramuscular	2	5	2	1	2	1.5	4	3		100
		24	1	1	2.5	0.5	0.5	0.5		100
		72	0.5	3.5	3	3	1	1		100
		144	>60	>60	>60	8	>60	>60		17
Percutaneous	2	5	16.5	4	>60	>60	4	>60		33
		24	7.5	1.5	2	5.5	1.5	21		83
		72	4.5	>60	1.5	>60	7.5	18.5		50
		144	>60	>60	>60	>60	>60	>60		0
Percutaneous	4	5	>60	>60	4	3.5	>60			40
		24	1.2	2	2.5	2.2	7.2			100
		72	8	>60	3	1.5	>60			60
		144	>60	>60	>60	>60	>60			0
Percutaneous	8	5	2	1.5	>60	14	14			40
		24	6	0.8	2.5	1.5	1.2			100
		72	3.5	5	11.5	31	1			60
		144	>60	>60	22	1	4			40
Percutaneous	16	5	4	1.5	6.5	2	40.5			80
		24	4.5	1	0.5	0.8	3			100
		72	1.5	2	1.5	1.5	3.5			100
		144	3	6.5	>60	2	31			60
Percutaneous	32	5	0.5	0.3	0.3	2	0.3	0.3	0.5	100
		24	1	0.3	0.8	0.5	0.2	1	0.3	100
		72	2.5	1	*	1.5	0.5	1.5	3	100
		144	3	1.5		2	4	2	2	100

*Died.

Each series of 4 clotting times determined at 5, 24, 72 and 144 hours after dosing and arranged vertically represents a different chick.

Chicks whose blood clots within 10 minutes or less are arbitrarily classed as cured.

The 2-methyl-1,4-naphthoquinone was dissolved in corn oil for the intramuscular injections.

5-hour, the 72-hour or the 144-hour periods, marked activity persists for a long time. Even at the 2 γ dose, the majority of the animals still showed low clotting times at the end of 3 days while the largest dose was remarkable not only for the very low clotting times observed at the 5-hour and 24-hour periods but also for the fact that they were still very low at the end of 6 days. The results of oral and intramuscular injection are also given in Table I.

Summary. The percutaneous route affords a means of conveniently administering slightly soluble materials possessing vitamin K activity. Doses as small as 1 γ per chick successfully cured approximately 50% of a series of depleted chicks. Larger doses afforded protection for relatively long periods of time.

12053

Quantitative Effects of Implantation of Cattle Anterior Pituitary Powder on Gonads of Immature Rat.

ROBERT H. SHULER. (Introduced by A. J. Carlson.)

From the Department of Physiology and Pharmacology, College of Pharmacy, University of Nebraska, Lincoln.

The use of subcutaneous or intramuscular "depots" for giving a more or less constant absorption of biological substances is a procedure of recognized value. Such slow, steady absorption should and perhaps does approach closely the same effect as glandular secretion within the body of the animal. This paper presents the results of the administration of cattle anterior pituitary powder* to rats by subcutaneous implantation.

The effect of implanting anterior pituitary powder on the ovaries of the rat was studied by Loeser[1, 2] and Janssen and Loeser.[3]

---

* The cattle anterior pituitary powder was furnished through the courtesy of the Geo. A. Breon and Co., Kansas City, Mo.

The author wishes to express his thanks to Dr. C. W. Sondern and Mr. A. W. Tandy, Jr., of the Geo. A. Breon and Co., and to Dr. H. G. O. Holck and Miss L. Mills of the University of Nebraska for their assistance and suggestions on this work.

Aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[1] Loeser, A., *Arch. Exp. Path. Pharm.*, 1930, **147**, 106.

[2] Loeser, A., *Arch. Exp. Path. Pharm.*, 1930, **148**, 377.

[3] Janssen, S., and Loeser, A., *Arch. Exp. Path. Pharm.*, 1930, **151**, 188.

They found newly developed corpora lutea after 96 hours. Attempts at quantitative evaluation were made on the basis of the number of rats showing luteal development, in relation to the dosage employed. Since their data showed that 50 mg total dose of powder gave significant results, this dosage was employed in our experiments. Organ weight was used as the basis for evaluation, since D'Amour and D'Amour[4] and others have found this to be the most sensitive, and adaptable to quantitative measurements.

Procedure. Acetone-dried cattle anterior pituitary powder had been molded into tablets containing 25 mg of the powder with about 20% milk sugar per tablet. These tablets were ether-sterilized previous to the experiments. The implanting procedure was as follows: Rats used were 22-24 days old at the beginning of each experiment. Each animal was weighed on the first, second, and fourth days of the test. First, the hair was clipped from the dorsal thoracic region. Under light ether anesthesia the clipped area was sterilized with 70% alcohol after which an incision 3-4 mm long was made in such a place as to be out of reach of the hind feet and the teeth. With a pair of blunt scissors a deep subcutaneous pocket was made ventrad to the incision. Into this pocket was placed a pituitary tablet, followed by 2 or 3 drops of sterile physiological saline solution. The incision was then dried with ether and covered with flexible collodion. Twenty-four hours later, another implantation was made in the same animal on the opposite side for the first incision. Controls were subjected to the same operative procedures, but no tablets of any kind were inserted. Ninety-six hours after the first implant the animals were killed; the thymus, testes, or ovaries and uterus were carefully removed, cleared of extraneous tissue, and placed in a modified Bouin's solution. Twenty-four hours later each organ was dried with blotting paper and weighed to the nearest milligram. This method of preserving organs and weighing the next day enabled the operator to use larger numbers of rats in a single experiment than would have been possible if the organs were weighed immediately after excision.

In all animals there was a small amount of sclerosing at the implantation site, but as far as could be determined this was due to the action of the concentrated lactose solution, and not to infection. For the short duration of the experiment, this deterioration of subcutaneous tissue was probably not detrimental to the animal as a whole.

---

[4] D'Amour, M. C., and D'Amour, F. E., *J. Pharm. and Exp. Ther.*, **1938**, **62**, 263.

Results and Discussion. A total of 58 rats was used, both hybrids and white Wistar-strain animals being tested in an effort to determine whether one strain was more responsive than the other. To make organ weights directly comparable, absolute weights were converted into milligrams per 100 g of animal. Although total body weights are not given in these data, one rather striking point was noticed. Of the 35 experimental animals, 25, or 71.5%, either lost weight or did not gain during the first 24-hour period of the experiment, while 91% (21 out of 23) of the control rats gained weight during the same period. Since the controls were operated on in exactly the same manner as the experimentals, this weight loss must have been due to the pituitary administration. With but two exceptions (both experimentals) all of the animals showed a gain in total weight by the end of 96 hours.

The accompanying table shows comparative results on organ weights. It is obvious that the white rats were more responsive than the hybrids. The critical ratio of the difference of weight

TABLE I.
Weights of Organs in Experimental and Control Rats.

	Experimental Avg.				Control Avg.				
Organ	No. of animals	Wt and range in mg	Wt in mg/100 g	Sigma	No. of animals	Wt and range in mg	Wt in mg/100 g	Sigma	Critical ratio
			Hybrids.						
Uterus	12	61 23-112	124	44.6	7	38 19-55	74	21.4	3.08
Ovaries	12	30 10-66	66	26.5	7	28 9-40	58	17.9	0.73
Thymus	11	117 70-215	266	62.3	7	162 98-259	332	50.7	2.60
			Whites.						
Uterus	14	56 32-127	188	104.4	9	25 19-33	78	19.7	3.69
Ovaries	14	19 9-32	61	18.8	9	13 9-20	40	7.72	3.58
Thymus (female)	14	60 26-105	189	59.6	9	117 72-170	345	73.8	5.03
Thymus (male)	6	69 53-102	201	38.0	5	110 98-148	297	50.1	3.48
Testes	6	233 196-290	648	47.7	5	178 164-234	484	48.8	6.13

Wts of animals in grams:

Hybrid	Experimental	48.3 (32-58)
	Control	47.7 (30-60)
White	♂ Experimental	34.2 (29-42)
	Control	37.2 (32-46)
	♀ Experimental	31.2 (23-44)
	Control	34.0 (27-45)

increase of testes of albino male experimentals and controls was 6.13, which, according to Fisher's tables,[5] is very significant. Likewise the differential responses of uteri and ovaries of white rats are very significant. Thus we may suggest that any of these organs would be useful in standardization procedures. One animal alone was responsible for the very large standard deviation of the single determination (sigma) upon the uteri. In this animal the uteri were very large, the cavity being filled with fluid (it was over 500 mg per 100 g).

The thymic response of female white rats gave a high critical ratio, but with a large standard deviation. This positive thymic response, however, may or may not be due to operative damage to the animal, as suggested by Selye[6] and Selye and Masson.[7] In any case, the thymic responses are possibly not a direct result of the action of pituitary hormones, but reflect hormonal activity of other endocrine glands.[8]

These preliminary experiments show that it is possible to obtain statistically significant responses to cattle anterior pituitary implants in white rats, using weights of testes, ovaries and/or uteri as the criteria of activity. Further work should be directed at determining the minimum amount of extract which will produce significant changes, and in attempting to make comparisons with other standardization procedures. It is interesting to note that the white rat, either male or female, is more sensitive to cattle anterior pituitary than to similar preparations from the sheep.[4]

Summary. 1. A method is described for administration of pituitary powder by the tablet implantation technic. 2. Statistically significant responses were obtained with 50 mg of powder in case of testes, uteri, and ovaries of 22- to 24-day-old white rats. 3. These responses were measured by organ weight gains rather than by histological changes or vaginal smears.

---

[5] Guilford, J. P., *Psychometric Methods,* McGraw, Hill and Co., 1936. (See Table K.)

[6] Selye, H., *Am. J. Physiol.,* 1938, **123**, 758.

[7] Selye, H., and Masson, G., *Endocrinology,* 1939, **25**, 211.

[8] Persike, E. C., Jr., *Am. J. Physiol.,* 1940, **130**, 384.

12054

Action of Desoxycorticosterone on Non-Protein Nitrogen Content of Blood During Experimental Uremia.*

HANS SELYE AND KAI NIELSEN.

From the Department of Anatomy, McGill University, Montreal, Canada.

Previous experiments in the mouse and rat showed that desoxycorticosterone in the form of its acetate exerts a beneficial effect during experimental uremia inasmuch as it prolongs the survival time following bilateral nephrectomy.[1] The question arose whether this effect is due to an increased resistance to uremia or whether desoxycorticosterone actually prevents the development of the uremic condition. In order to clarify this problem, we determined the N.P.N. of bilaterally nephrectomized desoxycorticosterone acetate (D.C.A.) treated rats simultaneously with that of nephrectomized but otherwise untreated controls. In order to eliminate the possibility of complicating the experiment by injury to the adrenals, the nephrectomy was performed following careful decapsulation of the kidney, a precaution which makes any adrenal injury highly improbable. As an added safety measure, the right kidney, which is especially close to the adrenal was removed several days before ablation of the left kidney. Thus even if there had been some interference with the right adrenal at the time of the first intervention, sufficient time was allowed for its recovery.

Twenty male albino rats weighing 55 to 100 g were divided into 2 equal groups of 10 animals in such a manner that the average weight was 87 g in both groups. In a first operation, the right kidney was removed in all animals. Three days later, subcutaneous injections with 10 mg of D.C.A. in 0.25 cc of peanut oil were given to the first group on 4 successive days, the second group remained untreated, acting as controls. Six days after the ablation of the right kidney, the left kidney was removed in all animals. Twenty-four hours after the second operation, all the control animals showed obvious signs of uremia (muscular weakness, tremor) and one animal succumbed, while the D.C.A.-treated group was still

---

* This investigation was supported by a grant from the Committee on Research in Endocrinology of the United States National Research Council. The author is greatly indebted to Drs. Gregory Stragnell and Erwin Schwenk of the Schering Corporation of Bloomfield, New Jersey, for the desoxycorticosterone acetate used for these experiments.

[1] Selye, Hans, *Canad. Med. Assn. J.*, 1940, **43**, 333.

in good condition. During the following 6 hours, 6 of the untreated controls went into deep coma and respiration became so shallow that it was obvious they were dying. Therefore, in order to obtain blood for N.P.N. determination (Folin and Wu method modified for microdetermination with the Evelyn photo-electric colorimeter) these animals were killed. Thirty-two hours after the second nephrectomy, the only 3 surviving controls were killed simultaneously with the D.C.A.-treated animals which were still in fairly good condition even at this time. The blood N.P.N. of the untreated controls varied from 180-268 mg %, average 204 mg %, while that of the D.C.A.-treated animals varied from 148-188 mg %, average 170 mg %, although most of the animals in the latter group were sacrificed later than the controls. The significance of this apparent difference between the treated and untreated animals was evaluated by "Student's" method for small samples and is expressed in terms of probability estimated by graphic interpolation in Fisher's table of t.[2] It is generally agreed that differences may be regarded as significant if P is smaller than 0.05 and in our experimental series, $P =$ less than 0.01, consequently the inhibition of the N.P.N. rise must be regarded as highly significant. This conclusion is supported by 2 additional experimental series which were carried out under similar circumstances and gave essentially the same results.

We feel that on the basis of these observations, one may say that D.C.A. tends to counteract the development of uremia in the nephrectomized rat. It remains to be seen whether this action is due to an inhibition of protein catabolism.

Summary. Experiments on rats indicate that treatment with desoxycorticosterone acetate not only prolongs the survival time and delays the clinical signs of uremia, but actually inhibits the rise in the non-protein nitrogen content of the blood after complete nephrectomy.

---

[2] Fisher, R. A., *Statistical Methods for Research Workers,* 6th ed., Edinburgh, 1936, p. 128.

12055 P

Form of the Reflex Response in Relation to the Pattern of Afferent Stimulation.

LOUIS L. TUREEN. (Introduced by G. H. Bishop.)

From the Laboratory of Neurophysiology and Department of Neuropsychiatry, Washington University School of Medicine.

The contraction curve and the after discharge of the flexor reflex response to selective ipsilateral afferent stimulation were studied on 52 cats. Action potentials of the afferent impulses in the popliteal or saphenous nerve, tibialis anticus muscle reflex in spinal cats were recorded. In this group of experiments, discharges from a small condenser (.001 mf) furnished the stimulus. Larger condensers (.02 mf) were used in a second group of experiments which included also decerebrate animals. It was established that with the larger condensers repetitive firing of groups of fibers of the afferent nerve followed a single shock; only the lower threshold fibers fired twice with small voltages. As the strength of stimulus was increased, more of the fibers were repeatedly stimulated, while the higher threshold fibers were fired once. Large voltages repeated the entire action potential with each shock. With the small condenser discharges, a single stimulus fired only once all fibers within the threshold of that stimulus. The rate of stimulation varied from 1 to 15 per second, and in most instances periods of stimulation did not exceed 10 seconds.

Results. Voltages within the threshold range of the large fibers of the A group, including the alpha, beta and gamma fibers, produced excitatory responses in the contraction curve which persisted during the period of stimulation at a constant level. Cessation of stimulation resulted in prompt relaxation of the muscle, without indication of an after discharge. Action potentials indicated that alpha fiber threshold coincided with 1.5 v at .001 mf. The rest of the alpha, beta, gamma fibers of the A group were fired with shocks of 3-4.5 v.

Stronger voltages (6 v) produced a slightly stronger contraction, which decreased after an initial maximum. An after discharge was manifest in the delay in relaxation of the muscle at the conclusion of stimulation. Progressively stronger voltages produced initially stronger contractions which, however, fell off more rapidly during stimulation, and were followed, after stimulation stopped, by after discharges, occurring as separate slow contractions, a latent period intervening. With 6 volts the lower threshold fibers of the delta wave discharged; with stronger stimuli, progressively more fibers of the delta wave were fired. The after discharge coincides with the stimulation of delta fibers, and its latent period becomes longer as the voltage increases.

Large condenser discharges resulted in variations of the contraction curve which could be interpreted in the light of repetitive firing of selective groups of fibers. Small voltages, particularly those near threshold, usually produced a contraction curve characterized by more or less fusion of individual contractions, and a persistent tendency to build up tension in the contraction curve. After discharges, if present, were immediate in type, with a very small latent period. As the voltage was increased, the contraction curve flattened out into a plateau, and with stronger stimuli the curve declined. After discharges showed a longer latent period as the stimulus was increased.

The build-up of tension is obviously the effect of excitatory fibers. The maintenance of a plateau is thought to indicate a balancing between excitatory and inhibitory stimuli. The decline in the contraction curve with strong stimuli is presumably caused by the preponderating effect of inhibitory impulses. The after discharge then appears because the inhibitory after effects cease more quickly than the excitatory after effects. The latency of the development of the contraction of the after discharge is due to the algebraic summation of inhibitory and excitatory after effects.

In these nerves the threshold for reflex contraction is not the threshold for the most excitable afferent fibers, but occurs near the maximum of the first wave in the action current record. Muscle tension increases throughout the range of delta fibers. Inhibitory effects are evident only with delta fiber stimulation. Therefore, while inhibition and excitatory ranges partially overlap, the large fibers tend to be excitatory, the small fibers inhibitory.

These conclusions are not inconsistent with the observations of Forbes, Davis and Lambert.[1]

---

[1] Forbes, Davis and Lambert, *Am. J. Physiol.*, 1930, **95**, 142.

12056 P

Rôle of Heart Sounds in Diagnosis of Experimental Endocarditis Lenta in Rabbits Before Death.*

JAN NYBOER, MARTHA JANE SPENCE AND WARD J. MACNEAL.
From the Department of Medicine and the Department of Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.

MacNeal and his coworkers[1, 2] have successfully transferred to rabbits the human strains of *Streptococcus viridans* obtained from active cases of subacute bacterial endocarditis. The vegetative lesions developed in rabbits on the cardiac valves, which however were not known to be previously injured or diseased. Other localizations of the infection were often present. The diagnosis of endocarditis before death was attempted on the basis of successively positive blood cultures, development of anorexia and marked loss of weight. These criteria were often inadequate, as proved by autopsy.

The difficulty in antemortem diagnosis of active experimental endocarditis has greatly hampered the therapeutic approach to this problem, which is admittedly an important one. In view of the large valvular lesions developed, often producing a severe stenosis, it was logical to assume the development of murmurs and modification of the heart tones. Therefore each animal was examined at frequent intervals, in unbiased procedures, by a stethoscope. Some were selected for a stethogram during the experimental period. The rapidity of the heart, the respiratory tones and the animal movements had discouraged this procedure previously. These objections were partly overcome during a mechanical asphyxia of the animal. A marked bradycardia and a prolonged apnea was usually produced by this procedure; the prolonged diastole aided the differentiation of the heart sounds. The results and correlations are summarized in Table I.

It is concluded that a study of the heart sounds is of distinct value in detecting experimental endocarditis in rabbits. Striking correlation

---

* Aided by a grant from the Oliver-Rae Fund of the New York Post-Graduate Medical School. Appreciation for the use of special equipment is expressed to Dr. Charles A. Poindexter.

[1] MacNeal, Ward J., Spence, Martha Jane, and Wasseen, Marie, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 473.

[2] MacNeal, Ward J., Spence, Martha Jane, and Wasseen, Marie, *Am. J. Pathol.*, 1939, **15**, 695.

TABLE I.

	Ante-Mortem findings Auscultation	Ante-Mortem findings Stethogram	Post-Mortem findings Cardiac changes and their correlation
Total rabbits studied	29	14	29
Definite murmur	4	3 of those positive on auscultation were also positive by the graphic study. One was not studied.	All 4 rabbits had large stenosing vegetations on the mitral valve.
Uncertain murmur	1	The same one was uncertain by graphic study.	This animal had a small vegetation about 1 mm in diameter on the mitral valve.
Transient murmur	1	No observation made.	This animal showed a slight roughening of the mitral cusps.
Gallop rhythm or modified sounds	1	This same one and also 3 others were positive in the graphic study. One of these 3 later developed a definite murmur and stenosing mitral lesion and is included in the Definite Murmur Group above.	The one animal positive on auscultation showed a large myocardial infarct of the left ventricle, but no vegetations on the valves. Another had small smooth vegetations on mitral and aortic valves. In one the heart was negative.
No murmur	22	7 of these were negative also by graphic study. The other 15 were not studied.	Of this negative group, one animal had two large smooth vegetations above the mitral valve, without stenosis; 4 had small vegetations on one or more valves; 4 showed gross changes in the papillary muscles; one had a mural endocardial lesion; 12 had negative post-mortem heart findings.

existed between auscultatory and stethogram findings when severe mitral stenosis was produced by the vegetations. Modified sounds usually occurring in presystole were not easily detected by auscultation but were recorded by the stethogram. Minimal lesions of the valves, papillary muscles, or mural endocardium were usually not detected by auscultation and recording of sounds from the apical precordial region.

12057

Non-Pathogenicity of Gonococci for Larger Animals.*

Ray E. Trussell and S. H. McNutt. (Introduced by E. D. Plass.)

From the Department of Obstetrics, State University of Iowa, and Veterinary Research Institute, Iowa State College.

The need for an animal naturally susceptible to gonococcal infection is well known. Practically all common laboratory animals have been inoculated by natural and unnatural routes and the results have been almost uniformly discouraging. The only lower animal known to be susceptible to the gonococcus is the white mouse and this nonimmunity can be shown only through intraäbdominal inoculation of relatively large doses suspended in mucin. The situation has been well summarized by Thomas and Bayne-Jones[1] and more recently by Thomas,[2] who attach considerable importance to the problem.

There appear to have been few efforts to study gonococcus inoculations in larger domestic animals. Stockmayer and Schmitz[3] inoculated young heifer calves intravaginally with fresh pus from cases of gonorrhea. Six of the 11 animals used developed mild vaginal reactions, but no intracellular cocci could be demonstrated by smears, no positive cultures were obtained and no positive complement-fixation reactions developed. In this connection it may be mentioned that many heifers will develop a mild non-specific discharge following intravaginal manipulation.

The data offered here summarize a series of attempts to infect 5 species of domestic animals by the inoculation of a pure culture of *Neisseria gonorrheæ* intravaginally or into the conjunctival sac.

Experimental. Three heifers, 5 female goats, 4 ewes, 2 sows, 1 mare, 2 bull calves, and 1 boar were utilized as experimental animals. The culture of gonococci used was derived from a case of acute pelvic inflammatory disease and had been maintained for one

---

* Part of a coöperative study carried out at the Veterinary Research Institute of Iowa State College. Sincere appreciation is expressed to Dr. Chas. Murray, Dean of the Division of Veterinary Medicine, Iowa State College, and Dr. E. D. Plass, Professor of Obstetrics and Gynecology, College of Medicine, State University of Iowa, for making this work possible.

[1] Thomas, R. B., and Bayne-Jones, S., *Am. J. Syph., Gonor., and Ven. Dis.*, **20**, Supplement, January, 1936.

[2] Thomas, R. B., *The Gonococcus and Gonococcal Infections*, Supplement No. 8 to Venereal Disease Information, United States Public Health Service, 1939.

[3] Stockmayer, W., and Schmitz, J., *Z. f. Immunitätsforsch. u. exp. Therap.*, 1935, **84**, 371.

month on chocolate-agar slants (Douglas agar + sheep's blood). Inoculations were made by saturating sterile swabs with a freshly prepared saline suspension of the organisms and inserting the swabs deep into the vaginas of the female animals or beneath one lower eyelid. The swabs were purposely applied vigorously and extensively to the mucous membranes. After the animals were inoculated the saline suspensions were cultured on chocolate-agar plates and all developed heavy growths of gonococci.

Cultures from the inoculated areas were made with sterile swabs every 2 to 3 days. Vaginal cultures from the larger animals were taken through glass or metal specula. Conjunctival cultures were secured by pulling down the lower eyelid and sweeping the swab the full extent of the sac.

The swabs were dropped into 1 cc of sterile broth which was protected from chilling and inoculated within one hour on freshly poured chocolate-agar plates. These were incubated 48 hours at 35-36°C under 10 to 12% CO_2. The plates were then examined for colonies morphologically suggestive of gonococci which gave a positive "oxydase" reaction. Suspicious colonies were isolated in pure culture for study.

Results. No gonococci were recovered from any of the inoculated animals. Many of the vaginal cultures from sows were badly overgrown with fecal "spreader" organisms. The sheep all developed bilateral purulent conjunctivitis associated with oxydase-positive organisms, but these bacteria formed more opaque colonies than gonococci and fermented all 4 test sugars. These animals had hair closely matted about the eyes and it is likely that the irritation produced by the hair and repeated swabbing produced the conjunctivitis. A few of the heifers developed a scant vaginal discharge, but no gonococci were recovered and the irritation probably resulted from the repeated insertions of the speculum.

Each animal was observed for 6 to 24 days after inoculation, during which interval from 4 to 7 cultures were made, except that one ewe was subjected to only one vaginal and to three conjunctival cultures. No smears were made since our experience[4] has paralleled that of others in that properly prepared cultures are superior. The culture-technics outlined by Carpenter[5] were used.

Summary. Vaginal and conjunctival inoculations of a pure culture of *Neisseria gonorrheæ* in cattle, sheep, swine, goats and the horse have failed to produce any infection demonstrable by repeated cultures.

---

[4] Tucker, W. W., Trussell, R. E., and Plass, E. D., *Am. J. Obst. and Gynec.*, 1939, **38**, 1055.

[5] Carpenter, C. M., *Am. Pub. Health Assn. Year Book*, p. 125, March, 1937.

Sincere appreciation is expressed for the help of John C. Carey and Corwin L. Smith in carrying out these experiments.

12058

Passage of the Blue Dye T-1824 from the Blood Stream into the Lymph.*

Joseph W. Ferrebee, Octa C. Leigh and Robert W. Berliner.
(Introduced by W. W. Palmer.)

From the Departments of Medicine and Surgery, Columbia University, College of Physicians and Surgeons, and Presbyterian Hospital, New York City.

The fate of intravenously injected Brilliant Vital Red has been discussed by H. P. Smith with special reference to its use in blood volume determinations.[1, 2] The following observations on the blue dye T-1824 are reported at this time because of the interest in blood volume and shock which has resulted from the present preoccupation with problems of military importance. Our purpose is to record the fact that the blue dye T-1824 passes from the blood into the lymph and to discuss the bearing which this observation has upon estimations of plasma volume by the Blue Dye Method.[3]

Method. Normal dogs were fasted overnight, anesthetized with morphine and nembutal, and the thoracic and cervical lymphatic ducts cannulated following the method of White, Field, and Drinker.[4] Samples of venous blood and of thoracic and cervical lymph were collected to serve as blanks for the subsequent dye determinations. One cc of a 1% solution of the blue dye T-1824 was then injected into the external jugular vein. In each instance solutions of sodium thiocyanate and in 2 instances solutions of horse and rabbit globulins specific for type III pneumococcus were also injected. Observations were made of the time at which these materials appeared in the lymph and the distribution which they underwent between the blood and the lymph.

---

* The writers wish to thank Dr. Magnus I. Gregersen, Department of Physiology, College of Physicians and Surgeons, for his help and the use of his laboratory and equipment in carrying out this study.

[1] Smith, H. P., *Bull. Johns Hopkins Hosp.*, 1925, **36**, 325.

[2] Smith, H. P., *J. Exp. Med.*, 1930, **51**, 379.

[3] Gregersen, M. I., Gibson, J. G., 2d, and Stead, E. A., *Am. J. Physiol.*, 1935, **113**, 54.

[4] White, J. C., Field, M. E., and Drinker, C. K., *Am. J. Physiol.*, 1933, **103**, 34.

The concentration of blue dye and thiocyanate in the thoracic and cervical lymph and venous plasma was determined with a Koenig-Martins spectrophotometer following the method described by Gregersen and Stewart for blood plasma.[5] The concentration of specific horse and rabbit globulins was estimated grossly by precipitin reactions carried out with samples of pneumococcus type III carbohydrate prepared by Dr. M. Heidelberger.

Results. In each of the 4 dogs studied the blue dye T-1824 made its appearance in the thoracic and cervical lymph sometime during the first hour following its intravenous injection, the exact time and amount seeming to depend somewhat upon the rate at which the lymph was flowing. Fig. 1 illustrates the quantitative relationships developed between the dye concentration in the thoracic and cervical lymph and the blood plasma in one of the dogs in which satisfactory

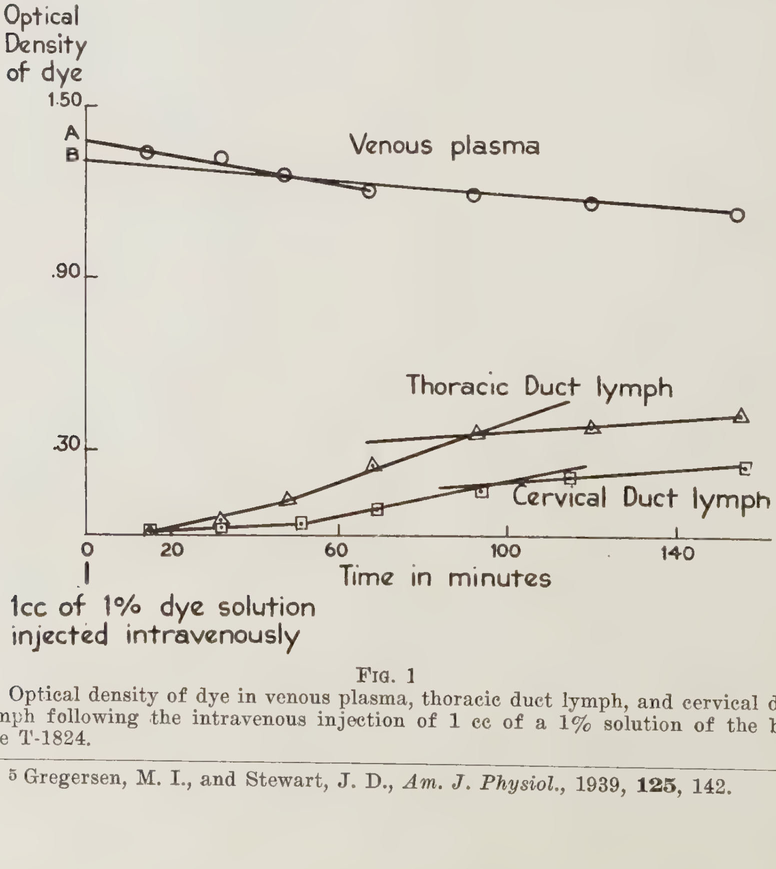


FIG. 1

Optical density of dye in venous plasma, thoracic duct lymph, and cervical duct lymph following the intravenous injection of 1 cc of a 1% solution of the blue dye T-1824.

---

[5] Gregersen, M. I., and Stewart, J. D., *Am. J. Physiol.*, 1939, **125**, 142.

collections of blood and lymph were possible over a considerable period of time. Similar results were obtained in the other animals.

It was found that the concentration of dye in the thoracic lymph considerably exceeded that in the cervical lymph, the values at 2 hours being of the order of 20 to 40% of the plasma concentration in the case of thoracic lymph and 3 to 15% of the plasma concentration in the case of cervical lymph. The volume of lymph collected also varied greatly but was of the order of 100 to 200 cc of thoracic duct lymph and 10 to 20 cc of cervical duct lymph per hour. In a dog such as that of Fig. 1 with a plasma volume of 600 cc these data indicate that approximately 7.5% of the injected dye† and 15% of the plasma proteins passed through the thoracic duct in the course of 2 hours.‡ How much more dye passed into the lymphatic system and remained there during this period cannot be estimated because the volume of the lymphatic system is not known.

The thoracic and cervical lymph of both animals receiving intravenous injections of horse and rabbit globulins gave strongly positive precipitin reactions for these foreign proteins at the end of 1 hour. A severe serum reaction occurred in one of the animals immediately following the injection of a toxic solution of rabbit globulin and it was interesting to observe that the urticarial wheals which developed on the abdomen were blue in color.

The concentrations of thiocyanate in the cervical and thoracic lymph and the blood plasma were found to be within 2 or 3% of one another at the end of 1 hour.

Discussion. Abell[6] has observed that in rabbits T-1824 is not visible in the extracellular tissue spaces of the ear during the first 2 hours following its intravenous injection.[6] Our experiments with normal dogs indicate that considerable concentrations of dye may be expected in the thoracic and cervical lymph during this period. The differences encountered are probably explainable on the basis that plasma proteins to which dye is attached circulate through the lymph, particularly through the thoracic duct lymph, even under relatively normal conditions. To what extent the circulation of dye through the lymph may be increased by damage to capillary membranes has not been determined. Maurer's experiments on lymph flow in anoxemia[7] would indicate that the increase may be considerable. As for the general extracellular fluid spaces, the increased escape of dye which follows capillary damage[8] is well illustrated by our dog with blue urticaria.

---

† Calculations:

Plasma vol. = 600 cc.

Lymph collected = 300 cc.

Conc. dye in lymph (mean during 2 hr period) $= \frac{0. + .30}{2} = .15$ plasma conc.

$300 \times .15 = 45$ or amount of dye equivalent to that in 45 cc plasma.

$\frac{45}{600} = 7.5\%$ of total amount of dye injected.

‡ Using, instead of mean concentration of dye, the approximate equilibrium concentration obtained at the end of 2 hours:

$300 \times .30 = 90$ or an amount of protein equivalent to that in 90 cc of plasma.

$\frac{90}{600} = 15\%$ of total plasma protein.

[6] Abell, R. G., *Anat. Rec.*, 1940, **78**, 215.

The simple fact that dye appears in the lymph during the 30 to 60 minutes required for a determination of plasma volume is not of critical significance in evaluating the accuracy of the Blue Dye Method. The important question is to what extent correction can be made for the dye losses by extrapolating the curve of dye disappearance. The fundamental problem here lies in distinguishing the mixing curve from the disappearance curve. Whipple's observations on the mixing time of labelled erythrocytes[9] appear to establish the validity of the assumption that the first 10 minutes of the curve represents mixing time and should be neglected in the extrapolation.[10] Linear extrapolation of the remainder of the curve may be and frequently is arbitrary (Fig. 1, intercept A or B?) but the uncertainty involved rarely exceeds 5%. Moreover, such evidence as is available indicates that estimations obtained by this method under normal conditions agree as well as can be expected[11, 12] with those derived by other technics.[13] Whether equally satisfactory measurements can be made in shock under abnormal conditions of capillary permeability, lymph flow and mixing time remains to be seen. It is obvious, however, that difficulties will be introduced by variations in capillary permeability[14] and pressure and consequent fluctuations in plasma volume during the period of measurement.[15] Presumably, significant determinations will be possible only during periods in which constancy of hematocrit and protein concentration indicates a fair probability of equilibrium in the exchange of fluid between the blood and extracellular spaces.

---

[7] Maurer, F. W., *Am. J. Physiol.*, 1940, **131**, 331.

[8] Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.

[9] Hahn, P. F., Balfour, W. M., Ross, J. F., Bale, W. F., and Whipple, G. H., *Science*, 1941, **93**, 87.

[10] Gibson, J. G., 2d, and Evans, W. A., Jr., *J. Clin. Invest.*, 1937, **16**, 301.

[11] Smith, H. P., Arnold, H. R., and Whipple, G. H., *Am. J. Physiol.*, 1921, **56**, 336.

[12] Fähraeus, R., *Physiol. Rev.*, 1929, **9**, 241 (esp. 262-266).

[13] Bazett, H. C., Sunderman, F. W., Doupe, J., and Scott, J. C., *Am. J. Physiol.*, 1940, **129**, 69.

[14] Peters, J. P., *Body Water*, Charles C. Thomas, Baltimore, Maryland, 1935, Summary of literature, p. 58.

Summary. 1. Blue dye T-1824, used in the determination of blood plasma volume, passes from the blood stream into the thoracic and cervical duct lymph of normal dogs within the first hour following its intravenous injection. 2. Under normal physiological conditions correction for the escape of dye from the vascular bed can probably be approximated by extrapolating the curve of dye disappearance. Under abnormal and varying conditions application of this correction may be difficult.

12059 P

Effects of Cyanide and Iodoacetate on Survival Period of Infant Rats.*

H. E. HIMWICH, J. F. FAZEKAS AND F. A. D. ALEXANDER.

From the Departments of Physiology and Pharmacology and Anesthesia, Albany Medical College, Union University, Albany, New York.

It has been demonstrated that the infant rat and dog (1-12 days of age) exhibit a tremendous tolerance to anoxia, hypoxia, nitrous oxide, carbon dioxide, cyclopropane, and hypoglycemia.[1] Among the factors permitting prolonged survival in the infant are (1) low cerebral metabolic rate, (2) poikilothermia, and (3) some anaerobic source of energy. In the present experiments we wish to present additional evidence on the third of these possibilities. Rats 1 day postpartum (6 g wt) were given 5 mg sodium cyanide subcutaneously and their survival time, as indicated by respiratory efforts, was noted. Adult rats (150 g wt) given the same dose of sodium cyanide were also studied.

Young rats can tolerate for a long period (50 min) an amount of sodium cyanide, 5 mg, which will cause death in the adult animal in about 10 minutes. Since sodium cyanide is known to inactivate

---

[15] Magladery, J. W., Solandt, D. Y., and Best, C. H., *Brit. Med. J.*, 1940, 248.

* Aided by a grant from the Child Neurology Research (Friedsam Foundation).

[1] Himwich, H. E., Alexander, F. A. D., and Fazekas, J. F., in press, *Proc. Am. Physiol. Soc.*, April, 1941.

the oxidase portion of the respiratory system it may be concluded that such huge doses of sodium cyanide inhibit the aerobic processes depending on heavy metal catalysis. The survival period of cyanide is practically the same as that observed in nitrogen. Experiments on excised cerebral tissue of infant and adult rats disclose that N/200 sodium cyanide inhibits 95% of the total oxygen consumption.

Iodoacetic acid, 1 mg, was injected simultaneously in other infant rats. After allowing 15 minutes for absorption half of the total number of injected rats were placed in an atmosphere of nitrogen and the other half retained as controls respiring air. The infant rats which received iodoacetate and were placed in an atmosphere of nitrogen exhibited a survival time of only 3 minutes. Adult animals in nitrogen survived for the same short period. The injected infants breathing air lived for 50 minutes (the duration of the experiment). It seems, therefore, that the inhibition of glycolysis by iodoacetate removes the source of energy which permits prolonged survival in the young. It has been repeatedly demonstrated that the iodoacetic acid inhibits the change from triosephosphoric to phosphoglyceric acid in the anaerobic cleavage of glucose.

Summary. The prolonged survival period of infant rats, despite inhibition of the cytochrome-oxidase system with sodium cyanide, demonstrates the function of an anaerobic source of energy. The rapid death after the injection of iodoacetic acid and exposure to an atmosphere of nitrogen suggests the anaerobic conversion of carbohydrate to lactic acid as the source of this energy.

12060

Oxygen Saturation of Arterial Blood in Jaundice Complicating Lobar Pneumonia.*

FREDERICK H. KING AND ALAN LESLIE. (Introduced by Harry Sobotka.)

From the Medical Services and Laboratories of the Mount Sinai Hospital, New York City.

Jaundice in varying degree was a reasonably common complication of lobar pneumonia in the pre-sulfonamide era. It was usually accorded serious prognostic import, since the mortality was

---

* Aided by a grant from the Linde Air Products Company.

far greater than in the non-jaundiced cases. Many theories have been advanced to explain this jaundice in lobar pneumonia. Some are obviously untenable, while others appear plausible, because they are supported by experimental data. It has been attributed by various authors to an associated gastroduodenitis, to disturbed intrahepatic circulation, to the breakdown of hemoglobin during red hepatization of the lung, and to the presence in the liver of an hemolytic diplococcus. All these theories have proved inadequate.

Examination of autopsy material occasionally revealed parenchymal degeneration of greater or lesser extent. A more frequent finding was cholangiolitis. Both of these histologic findings seemed inadequate to explain the icterus, especially since they were observed in non-icteric cases as well. Klemperer and Gerber,[1] reviewing the pathologic findings in the liver in cases of lobar pneumonia, failed to find cholangiolitis consistently enough to attribute the jaundice to it alone.

The observation has repeatedly been made that in severe anemias, such as pernicious anemia, with consequent tissue anoxia, the liver may manifest central necrosis of varying extent with associated disturbance of function. Rich[2] demonstrated that rats placed in low-oxygen-tension chambers develop a diminished ability to excrete intravenously injected bilirubin, and that the livers of these animals show degeneration of cells in the region of the central veins. He proposed the theory that the anoxemia in lobar pneumonia, by its detrimental effect on the liver, renders this organ less efficient in the excretion of circulating bilirubin, which is frequently increased. This combination of hyperbilirubinemia and impaired ability of a liver, damaged by anoxia, to excrete the pigment effectively would seem to constitute the essence of the pathological physiology of jaundice complicating lobar pneumonia.

Twenty-nine cases of lobar pneumonia of various types were studied. Brachial artery punctures were performed and determinations were made of the oxygen content, capacity and percentage saturation of the arterial blood by the method of Van Slyke and Neill.[3] Immediately following the arterial punctures, bilirubin excretion tests were performed according to the technic of Eilbott,[4] as modified by Soffer and Paulson.[5] In this manner the initial blood level was determined, as well as the percentage retention, 4 hours after the intravenous administration of a predetermined amount of the bilirubin. Five percent retention of bilirubin after 4 hours was considered the upper limit of normal. Notation was made of the sputum type, the presence or absence of positive blood cultures, the hemoglobin at the time of the blood determinations, and of such pertinent therapy as might conceivably affect the data. In instances of jaundice, the bilirubin excretion test was omitted because of its unreliability.

---

[1] Klemperer, P., and Gerber, I., personal communication.

[2] Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1930, **47**, 338.

[3] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

[4] Eilbott, W., *Z. f. klin. Med.*, 1927, **106**, 529.

[5] Soffer, L. J., and Paulson, M., *Am. J. Med. Sci.*, 1936, **192**, 535.

There was no obvious correlation between the degree of anoxemia and the ability of the liver to excrete intravenously injected bilirubin. One case, the most anoxemic in the series, with an arterial blood oxygen saturation of only 73.5% showed no bilirubin retention. Another, with an oxygen saturation of 85.4% showed a retention of 5.8% which represents the slightest impairment, the figure being very close to the upper limit of normal. On the other hand, in another case, in which only moderate anoxemia obtained, the arterial saturation being 88.3%, there was a bilirubin retention of 15.4%. Still another, which had a bilirubin retention of 10%, had a normal oxygen saturation of 95.4%. A case showing marked bilirubin retention (12.1%) had only moderate oxygen unsaturation (90.1%). Other cases with impaired bilirubin excretion, less marked than those mentioned above showed little or no oxygen unsaturation; conversely, cases with more marked anoxemia showed little or no impairment of bilirubin excretion. Trend graphs, with the figures for both oxygen content as well as percentage saturation, plotted against blood bilirubin and bilirubin retention, showed no apparent relationship between these variables.

Many of the patients had received initial doses of sulfapyridine before the tests were performed. On the basis of experience with the action of this drug, it is believed unlikely that the blood determinations were at all influenced by these small initial doses of sulfapyridine.

Summary. (1) Twenty-nine cases of lobar pneumonia were studied with a view to determining any relationship between anoxemia and jaundice in this disease. (2) No obvious relationship could be demonstrated between anoxemia and the presence of jaundice or disturbed liver function as determined by the bilirubin excretion test.

12061 P

Activity in the Optic System Following Stimulation by Brief Flashes of Light.*

G. H. Bishop and S. Howard Bartley.

From the Laboratory of Neurophysiology, Washington University School of Medicine, St. Louis, Mo.

Details of the response of the optic nerve of the rabbit to stimulation by short light flashes, described in a previous paper, are also exhibited in the cat. The most obvious difference is that the cat record is less diagrammatic, more confused by apparently random activity. The cat retina is considerably the more sensitive as measured by the size of nerve responses to weak illumination. Immediate and delayed "on" responses are recognizable, each involving repetitive firing to the shortest stimuli. A distinct "off" response is produced by a flash of 50 msec, similar in form to the first "on". With much shorter flashes, an "off" response can be recognized superposed upon the second "on" discharge.

Records from the cortex and superior colliculus following flash stimulation of the retina have been correlated with the nerve response. The successive spikes of the nerve response, occurring at 100 per sec with strong light, show facilitation at the geniculate level, and the first few can be correlated with specific spike-like elevations in the cortical or colliculus record. When the first spike of the nerve record is the highest, the second spike in the cortical record may be higher than the first, presumably due to temporal summation within the 20msec period in which such summation occurs at the geniculate level after electrical shocks. The 3 nerve bursts, 2 "on" and one "off", are duplicated at the higher levels after suitable duration of flash.

In the cat, the major response of the colliculus following electrical shocks to the nerve is a slow wave activated by the highest-threshold fibers, in contrast to the cortex which is activated almost exclusively by the lowest-threshold units. The colliculus of the rabbit shows 2 successive elevations, one due to low-threshold and one to high-threshold fibers. In both animals these waves, which are postsynaptic, reverse in sign as a recording electrode passes from the surface of the colliculus downward, at about the level of the stratum

---

* Recipient of grants-in-aid from the Rockefeller Foundation and from the Committee on Benevolence of the Scottish Rite Masons for Research in Dementia Precox.

opticum. In the rabbit the 2 elevations present reverse at slightly different levels. This enables one so to place an electrode that to electrical stimulation these elevations are of opposite polarity.

An electrode thrust into the optic nerve back of the eyeball may be employed for electrical stimulation and recording, without serious interference with retinal stimulation by light. At a position of the recording electrode in the colliculus where the 2 elevations are of opposite sign with electrical stimulation, there also occur elevations of opposite sign after strong light flashes, enabling one to identify indirectly the firing of the different groups of fibers in the nerve activated *via* the retina. As the flash becomes weaker, the large-fiber response decreases before the small-fiber wave is materially affected. A specific interpretation of this and similar events is postponed until certain other characteristics of records obtained from the colliculus are better understood, but the prospect is that events in the retina and in the central nervous system may be correlated through the common denominator of fiber size, as detected by threshold to electrical stimulation.

12062 P

Reduction of Blood CO after X-ray Treatment.*

John A. Cameron. (Introduced by M. M. Ellis.)

From the Department of Anatomy, University of Missouri Medical School, Columbia, Mo.

Rapid recovery of rats[1] and monkeys[2] from severe CO effects when they were exposed to relatively small amounts of X-rays, has been previously reported. The X-rayed animals were restored to normal within a few minutes while their controls either died without regaining consciousness or exhibited a slow and laborious recovery.

This report presents the actual CO content of the blood of the treated pairs of rats in a new series of experiments. Albino rats of the Wistar stock were paired to give like ages and weights. Only male rats were employed but previous work has indicated no difference between the sexes in response to X-ray after CO.

---

* Aided by a grant from the University of Missouri Research Council.

[1] Cameron, John A., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 29.

[2] Cameron, John A., *Radiology*, 1941, in press.

Members of a pair were simultaneously exposed to 25% CO in air and removed from the gas chamber when it was judged the limit of toleration had almost been reached. Experiments in which the control rat died from over-exposure to CO were rejected, since it was necessary to analyze the living blood of both members of the pair. Immediately after removal from the gas chamber the animals were placed in glass trays, ventral side up and one, chosen at random, was placed, unscreened, under an X-ray tube delivering 125 r per minute at the target distance used, 10 inches.† Each X-rayed rat was exposed for the same number of minutes that the animals had been left in the CO chamber; usually 6 minutes CO was followed by 6 minutes X-ray, while the control rested in his tray.

Immediately following the X-ray exposure carotid blood from each animal was analyzed with a Hilger-Spekker spectrophotometer and a Hilger medium quartz spectrograph by Dr. Victor Ells, who calculated and evaluated the data obtained.

The quartz vessels used held a mixture of .07 cc blood in 75 cc citrate solution, and allowed the beam to pass through 1 cm of the fluid. Since only one blood sample could be placed in the spectrophotometer at any one time, either the X-rayed or control blood was subject to a delay in analysis of 4 to 5 minutes. To determine whether this delay had an appreciable effect on the CO content the control blood was examined first in the odd numbered experiments, the X-rayed blood first in the even numbered experiments. No difference in results was noted in relation to the order of examination. Seven experiments were completed with satisfactory spectrophotographs of the blood of both animals and of normal (untreated) animals. Three of the 7 were odd numbers (control blood examined first) and 4 were even numbers (X-rayed blood examined first). In each case the clinical indications were as expected, the X-rayed rat actively restored and the control rat in great distress. In each the spectrophotographs indicated a significant difference (5 to 15 times the probable error) between the CO content of the X-rayed and the control blood. Fig. 1 is a graph of experiment 7 showing the relatively high CO content of the control blood.

It appears from this study that the recovery of X-rayed animals from CO effects is not due to stimulation, increased resistance, or release of reserves, unless these factors themselves are responsible for the observed changes in the CO content of the blood.

The improvement shown by the X-rayed animals may be due to

---

† The X-ray machine and the services of the X-ray technician were kindly supplied by Prof. L. J. Stadler.

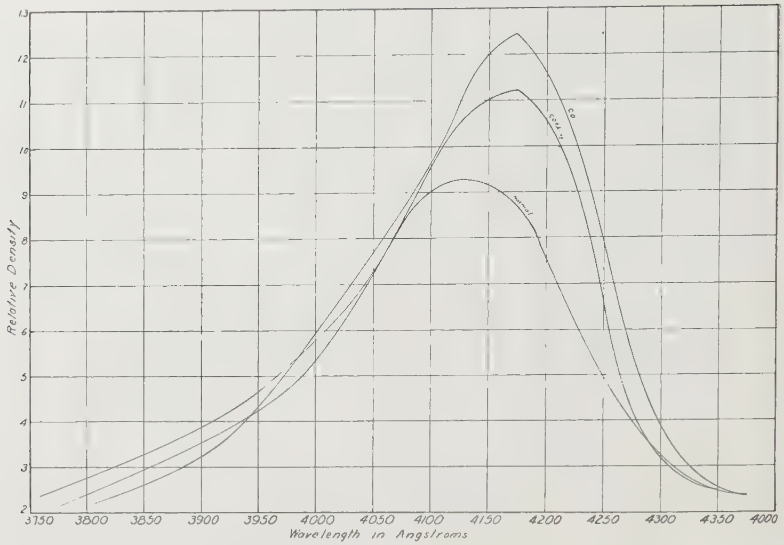


FIG. 1.

the combined effects of (1) X-ray dissociation of CO from blood cells; (2) deeper and more effective ventilation during X-ray exposure; (3) release of additional red cells into the blood stream due to X-ray effects.

12063

Distribution of Cholesterol Between Blood Cells and Plasma in Rabbit and Man.

SAMUEL MEMBER, SYLVIA B. EHRLICH AND MAURICE BRUGER.

From the Medical Research Laboratory, Department of Medicine, New York Post-Graduate Medical School and Hospital, Columbia University, New York.

Sperry and Schoenheimer[1] have shown that oxalated blood plasma of humans contained significantly smaller amounts of cholesterol than serum from the same blood sample. Schmidt[2] had previously recorded the same observtion for the phospholipids and had ascribed the difference to the fact that oxalate increased the plasma volume by abstracting water from the blood cells. As will be seen later, our observations confirm those of Sperry and Schoenheimer[1] but fail to agree with those of Chamberlain,[3] who maintained that in human blood the amount of cholesterol present in serum and whole blood were approximately equal.

---

[1] Sperry, W. M., and Schoenheimer, R., *J. Biol. Chem.*, 1935, **110**, 655.

[2] Schmidt, L. H., *J. Biol. Chem.*, 1935, **109**, 449.

[3] Chamberlain, E. N., *J. Physiol.*, 1928, **66**, 249.

Our interest in this problem was stimulated by the finding that in normal rabbits the cholesterol content of the serum was distinctly lower than that of oxalated whole blood, whereas the opposite was true for the cholesterol-fed rabbit. These observations confirm the results obtained by Dewey[4] and Chamberlain[3] for the normal rabbit but disagree with the finding of Dewey[4] that the cholesterol-fed rabbit behaves like the normal rabbit as regards the concentration of cholesterol in whole blood and serum.

Three types of experiments were carried out. In the first of these, 14 normal, healthy rabbits were used as controls. Blood was drawn from the ear vein and divided into two portions. One portion was allowed to clot spontaneously, while the other was treated with an amount of potassium oxalate just sufficient to prevent coagulation. In the second experiment, 28 rabbits were fed 3 g of cholesterol per week for 6 to 10 weeks. Blood samples removed at the termination of the cholesterol feeding were divided into two portions as described above for the control group. In the third series, oxalated whole blood, plasma and serum were obtained from 8 human subjects and the cholesterol content of each determined. In each instance, hematocrit determinations were also made and the cholesterol content of the blood cells calculated. A modified Sackett[5, 6] procedure for the determination of cholesterol was used throughout.

Table I indicates the results obtained.

Summary. In normal rabbits, the cholesterol content of whole blood is distinctly higher than that of serum or oxalated plasma prepared from the same blood sample. The blood cells of normal rabbits contain almost 2½ times as much cholesterol as an equal volume of plasma. In cholesterol-fed rabbits, the cholesterol content of whole blood is definitely lower than that of serum or oxalated

TABLE I.

Experiment	No.	Mean cholesterol (mg/100 ml) Whole blood	Plasma	Serum	Blood Cells*	Mean Hematocrit, %
Normal rabbits	14	121	87	82	194	32
Cholesterol-fed rabbits	28	514	628	694	189	26
Humans	8	212	227	279	193	43

*Calculated.

---

[4] Dewey, K., *Arch. Int. Med.*, 1916, **17**, 757.

[5] Sackett, G. E., *J. Biol. Chem.*, 1925, **64**, 203.

[6] Mirsky, I. A., and Bruger, M., *J. Lab. and Clin. Med.*, 1932, **18**, 304.

plasma. Cholesterol feeding does not influence the cholesterol content of the blood cells. Humans are unlike normal rabbits but simulate cholesterol-fed rabbits in the distribution of this sterol between cells and plasma. Cholesterol feeding produces an anemia in rabbits, an observation previously noted in the guinea pig by Okey and Greaves.[7]

12064

Bone Development in the Albino Rat on a Low Manganese Diet.

LeRoy L. Barnes, G. Sperling and L. A. Maynard.
From the Laboratory of Animal Nutrition, Cornell University, Ithaca, New York.

Studies by Norris and coworkers[1, 2, 3] have demonstrated the importance of manganese for the normal development of bone in the chick. The present study was undertaken to ascertain whether any similar bone abnormality could be noted in the rat. The low manganese diet used was the one employed by Daniels and Everson[4] with the exception that pasteurized milk was used instead of boiled milk and that cod liver oil was given separately once a week.

Sixteen females and 5 males raised from 21 days of age on this diet have shown as good growth as control animals on our stock diet. X-ray pictures of the right hind legs of these animals have been taken at regular intervals. No abnormalities have been observed in the tibias and no significant difference has been demonstrated between the growth of these tibias, as measured from X-ray photographs taken at intervals, and of the tibias of rats on the stock diet.

Five of the females raised on the low manganese diet were bred, using males reared on our stock diet. In the course of the next few months these females bore 57 living and many dead young. As would be expected from the work of others[4, 5] we were able to rear only a few of these. Sixteen of the 57 lived to an age of 100 days or more, and 13 of them were still alive at 300 days of age. Of the 16 here mentioned, 2 had abnormal tibias. These rats were both females. One of them was reared on the low manganese diet, and a series of X-ray pictures of its right tibia is shown in Fig. 1 (B). Paired with the tibia pictures of this rat, for comparison, are tibia pictures (A) of a female reared on the same low manganese diet, but born from a mother receiving a normal diet. Tibia B is abnormal in at least 3 respects. It is short. The epiphysis at the proximal end is thin. The diameter of the tibia at its proximal end is large in proportion to its length.

---

[7] Okey, R., and Greaves, V., *J. Biol. Chem.*, 1939, **128**, lxxvi.

[1] Wilgus, H. S., Jr., Norris, L. C., and Heuser, G. F., *J. Nutrition*, 1937, **14**, 155.

[2] Caskey, C. D., and Norris, L. C., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 590.

[3] Caskey, C. D., Gallup, W. D., and Norris, L. C., *J. Nutrition*, 1939, **17**, 407.

[4] Daniels, Amy L., and Everson, Gladys J., *J. Nutrition*, 1935, **9**, 191.

[5] Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1931, **92**, 651.

The other female showing the same abnormalities of the tibia at 23 days of age, was raised on the same diet supplemented with 1.5 mg Mn ($MnCl_2 4H_2O$) per 100 cc of milk. Successive X-ray pictures of its right tibia during growth showed a partial recovery from these abnormalities.

The body weights of these 2 animals showing abnormal tibias were below normal, so that the tibia lengths were not small in proportion to the body weight. In our experience with tibia pictures of several hundred rats on various diets, these 2 cases showing abnormal tibias are unique.

Fig. 2 shows curves of average tibia growth for rats, all of which were reared on the low manganese diet. At 300 days of age the average tibia length for the 6 females from mothers on a normal diet was

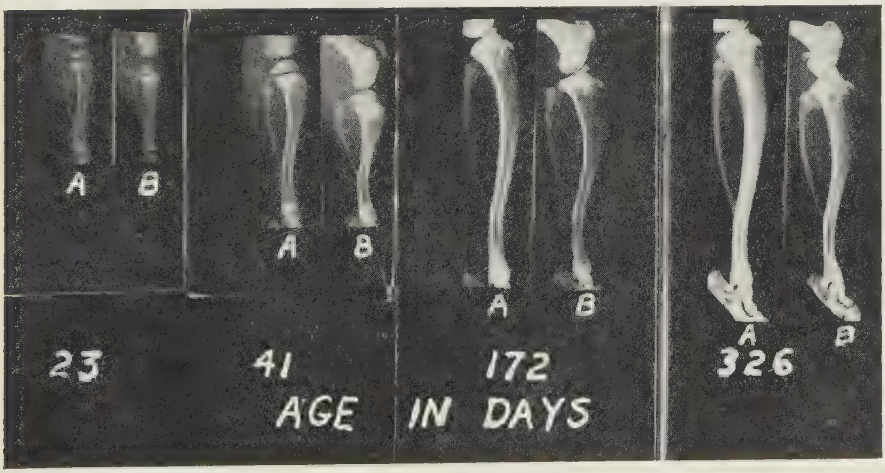


FIG. 1.

X-ray pictures of the tibias of two rats reared on a low manganese diet. A. Tibia of rat from mother on normal diet. B. Tibia of rat from mother reared on manganese-low diet.

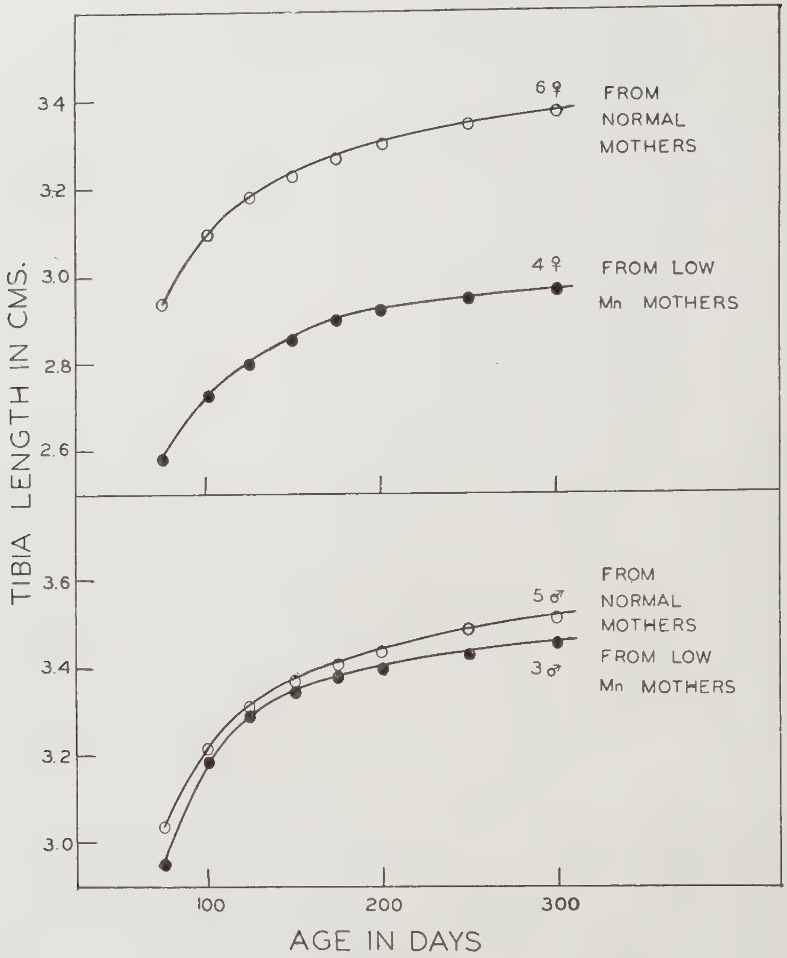


FIG. 2.
Tibia growth of rats reared on a low-manganese diet.

3.38 ± 0.06 cm, and for the 4 females from mothers on the low manganese diet 2.97 ± 0.11 cm. For the 5 males from normal mothers, the average tibia length at 300 days was 3.52 ± 0.05 cm, and for 3 the males from low manganese mothers 3.46 ± 0.04 cm. One is hardly justified in calculating probable error with such small numbers of animals, but the above figures do suggest that with respect to tibia growth, the female may be sensitive to a manganese deficiency in the mother's diet, while the male may not. It should be noted that these experiments were carried out before the rôle of an organic factor in perosis was announced[6] and thus no consideration was given to such a factor in our work. The average weight of the females from normal mothers was 25% higher at 300 days of age than the average weight of the females from low manganese mothers. In the case of the males there was very little difference in the average weights.

---

[6] Hogan, A. G., Richardson, L. R., and Patrick, H., *J. Nutrition*, 1940, **19**, Proc., 14.

Summary. We have not been able to demonstrate any abnormal tibia development in the albino rat raised on a diet low in manganese, provided the rat is normal at 21 days of age. In this respect the rat differs from the chick, at least in being much less sensitive to a low manganese diet. Only 2 cases of abnormal tibias have appeared in a total of 16 rats, born of females reared on a low manganese diet. Our limited data on rats from females reared on a low manganese diet suggest that the growth of the female is impaired, while that of the male is not.

12065

Relationship of Pantothenic Acid and Inositol to Alopecia in Mice.

D. W. WOOLLEY. (Introduced by D. D. Van Slyke.)

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

It has recently been shown in two laboratories[1, 2] that mice become hairless over large areas of the body when they are maintained on a purified diet. It has also been shown[3, 4] that inositol is a curative agent for the alopecia. With the isolation and identification of the anti-alopecia factor, it became of interest to study pantothenic acid deficiency in the presence of all other known essentials and also to determine if there were other unknown vitamins required by mice. It was observed that even in the presence of inositol, but in the absence of pantothenic acid, alopecia developed. The present report deals with alopecia which results from a lack of pantothenic acid and with that which results from a lack of inositol.

A highly purified basal diet which contained no unknown water-soluble constituents was prepared as previously described.[5] Panto-

---

[1] Woolley, D. W., *J. Biol. Chem.*, 1940, **136**, 113.

[2] Norris, E. R., and Hauschildt, J., *Science*, 1940, **92**, 316.

[3] Woolley, D. W., *Science*, 1940, **92**, 384.

[4] Woolley, D. W., *J. Biol. Chem.*, in press.

[5] Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 352.

thenic acid in the form of *d,l* sodium pantothenate[6] and inositol were added in the amounts desired for various experiments. Since only one optical isomer of pantothenic acid is biologically active, the levels of racemic acid quoted throughout this paper must be divided by two in order to obtain the quantity of active material which the animals received. General care of the mice was similar to that previously described.[1]

For the study of pantothenic acid deficiency the basal ration was supplemented with 100 mg of inositol per 100 g of ration. For the positive controls, it was further supplemented with 5 mg of pantothenic acid per 100 g. The first sign of pantothenic acid deficiency was retarded growth. After about 3 weeks on the experimental diet, the deficient animals became hyperirritable. A few days later they displayed difficulty in controlling their hind quarters. They seemed to be seized by periodic spasms of pain, for at intervals very violent and rapid movement would take place and the animals would squeal occasionally, as if in pain. The hind quarters sometimes exhibited convulsive, circular movements. After a few days the violent movements became subdued and a more or less complete paralysis of the hind legs ensued. In addition, the eyes of many of the animals were affected and in some cases they were unable to open them. A few days following the appearance of these symptoms, alopecia began to develop on the ventral surface of the body. The hair was lost first just behind the fore limbs on the chest. The denuded areas spread slowly over the entire ventral surface and then up the sides, and finally across the back. Some animals became completely naked except for the head. All of these symptoms were frequently not observed in the same animal. For example, the nervous and muscular manifestations were not invariably followed by alopecia, and alopecia sometimes developed without the appearance of the other symptoms. Occasionally animals died during the severe early manifestations and hence did not survive long enough to lose their hair.

When pantothenic acid was administered to animals at any stage of these conditions, a slow return to normality resulted. The violent movements could be eliminated within 1 or 2 days following the administration of 5 mg of pantothenic acid per 100 g of ration. The effect on alopecia was manifest much more slowly and it was usually necessary to continue treatment for about 3 weeks before restoration of hair began. Increasing the dosage of pantothenic acid up to 1 mg per day did not reduce this period. Eventually, however, the animals assumed a normal appearance.

---

[6] Woolley, D. W., *J. Am. Soc.*, 1940, **62**, 2251.

The symptoms preceding alopecia suggested those observed in pigs[7] and the syndrome of running fits in dogs.[8] It will be of interest to test the effect of pantothenic acid in these conditions.

The amount of pantothenic acid which the mouse requires cannot be stated with accuracy. Some indication of the minimum level was found in a series of experiments in which graded doses were given. Definite symptoms of pantothenic acid deficiency (effects on hind quarters, ventral alopecia) were noted with 500 γ per 100 g of ration. A few cases were observed following administration of 1 mg per 100 g. With 2 mg or 5 mg levels, no symptoms were observed and growth was satisfactory.

In the presence of adequate pantothenic acid and in the absence of inositol, alopecia resulted. For these studies the basal ration was supplemented with 5 mg of pantothenic acid per 100 g. After mice had been fed this ration for about 6 weeks, many of them began to lose their hair. The first bare spots were usually seen on each thigh, well up towards the spine. The hairless areas spread rapidly up the back and over the sides. The ventral surface was not affected. Usually the last areas to become affected in pantothenic acid deficiency were the first to show a lack of inositol, and vice versa. The difference in affected areas was usually most strikingly apparent in the early stages of the disease.

Administration of inositol to such animals resulted in regeneration of the growth of hair, usually about 10 days after therapy had been started. Soon the animals presented a normal appearance. It was observed that when such hairless animals were continued without inositol many of them regained their hair; only a very few lost weight and eventually died. For example, in a group of 13 hairless mice, 5 regained their hair within 3 weeks after the onset of the disease, one died, and 7 remained hairless throughout a period of 6 weeks. Some of the animals which had presented spontaneous cure did not exhibit a normal appearance but rather appeared unkempt and somewhat greasy. Such animals could be restored rapidly to a normal appearance by the administration of inositol.

Animals which received both inositol and pantothenic acid grew more rapidly than did those which received only pantothenic acid. With large doses (5 mg per 100 g of ration) of pantothenic acid the increment of growth produced by inositol was not large, but repeated trials have demonstrated that it was actual. For example, in 3 trials in which a total of 40 animals were employed, the average weights after 7 weeks with no inositol were 27 g, 33 g, and 24 g, whereas with inositol the weights were 26 g, 39 g, and 31 g. The average starting weights were the same.

---

[7] Chick, H., Macrae, T. F., Martin, A. J. P., and Martin, C. J., *Biochem. J.*, 1938, **32**, 2207.

[8] Arnold, A., and Elvehjem, C. A., *J. Am. Vet. Med. Assn.*, 1939, **95**, 303.

It was evident that both pantothenic acid and inositol influenced the growth of hair in mice. From the description given by Norris and Hauschildt,[2] it appears that their animals were suffering from a multiple deficiency. The effect on growth of pantothenic acid deficiency was much more pronounced than was that of inositol deficiency. (Fig. 1.) Deaths were much more common in the absence of pantothenic acid than in the absence of inositol. The fact that hairless animals receiving pantothenic acid but no inositol in many cases eventually regained their hair raises the question as to what should be called the "anti-alopecia factor". In the presence of inositol, no alopecia developed whereas in its absence hair was lost. Whether or not the effect of inositol was merely to increase the food consumption of the animals cannot be decided because of the impossibility of determining accurately the food consumption of mice.

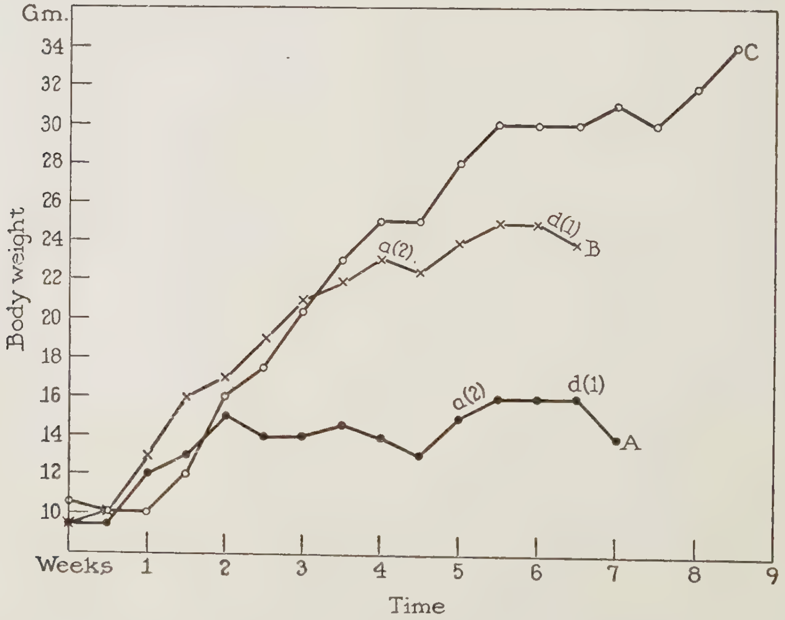


FIG. 1.

Growth responses to pantothenic acid, inositol, and pantothenic acid and inositol. Curve A represents the average response of 3 mice receiving inositol; Curve B represents the average response of 5 mice receiving pantothenic acid; Curve C represents the average response of 3 mice receiving both inositol and pantothenic acid. a = alopecia; d = dead; numbers in parentheses represent the number of animals affected.

TABLE I.
Incidence of Alopecia on Various Purified Rations.

Addition to 100 g of basal	Total No. of animals	No. of cases of alopecia
100 mg inositol	9	6
5 mg *d,l* pantothenate	39	9
5 mg *d,l* pantothenate and 100 mg of inositol	12	0
5 mg *d,l* pantothenate following a 2-week depletion period	35	19

It seems, however, that the inositol effect was not a mere reflection of pantothenic acid effect because of the difference in the early picture of alopecia in the two deficiencies.

The fact that mice may be raised satisfactorily and rapidly to maturity on a highly purified diet is noteworthy. The only constituents of the ration about which there can be uncertainty as to chemical composition are the oils and the casein; all the other constituents were pure compounds. It will be of interest to determine whether the oils and casein can be replaced by pure fat-soluble vitamins and pure amino acids. Whether or not natural foodstuffs contain materials which increase slightly the rate of growth obtained with the purified diet has not been determined.

12066

Rôle of the Intestinal Bacteria in the Decomposition of Pectin.

S. C. WERCH, A. A. DAY, R. W. JUNG AND A. C. IVY.

From the Departments of Bacteriology, and Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.

Previous observations[1, 2] have shown that orally administered pectin is decomposed in the human and dog gastrointestinal tract, that the decomposition occurs in the colon, and that it is probably brought about by bacterial action. A thorough study of the part played by intestinal bacteria in pectin decomposition has not as yet been made. Schneider,[3] however, found that mixtures of intestinal bacteria destroy the hemocellulose of apple marc. He also observed that reducing sugars did not constitute part of the end products.

---

[1] Werch, S. C., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 336; *Am. J. Dig. Dis.*, in press.

[2] Kertesz, Z. I., *J. Nutrition*, 1940, **20**, 289.

[3] Schneider, E. C., *Am. J. Physiol.*, 1912, **30**, 258.

The related work of McCoy and Peterson[4] is also of interest. They found that laboratory cultures of retting organisms may not ferment pure pectin under artificial conditions, and that pectin must be of known purity in order to avoid false reactions.

The pectin used in this study was pure citrus pectin obtained from the Research Laboratories of the California Fruit Growers' Exchange. It is essentially free of such impurities as pentoses, pentosans, color and other materials frequently found in other pectins. Before much work was done it became evident that a sterile pectin-broth medium was obligatory, and had to conform to the following requirements: (a) that the pectin be unchanged, (b) that the pH of the medium be readily set and adjusted within the range where pectin is relatively stable for the period of incubation, and (c) that it be easily sterilized.

Heat and chemical sterilization altered the properties of the pectin, and desiccation proved inefficient. Berkefeld filtration proved satisfactory, when certain precautions were observed in the care of the candle and pH of the medium. After being used, the candle is washed freely under tap water with a soft brush, and then rinsed in the reverse direction for 30 minutes (using gravity). Next it is placed for 10 minutes in a boiling water bath containing 5% $NaHCO_3$. After this it is rinsed with 3 liters of distilled water in the usual direction by means of suction. Lastly, slow drying is permitted by leaving it near a draft over night. The day it is to be used, the candle, mantle and suction flask are assembled, wrapped in a large muslin sheet and autoclaved for an hour. This procedure establishes a neutral or weakly positive surface charge on the candle, permitting a pectin solution of pH 7.2 (for example) to pass through, yet retaining the bacteria. The technic developed for the preparation of the pectin-broth medium conforms with our other requirements. Pectin is dissolved in a buffer solution of desired pH, and then added to a volume of sugar-free broth sufficient to make the desired dilution. The mixture may be adjusted for pH and then sterilized by filtration. Table I represents an analysis of such a medium, of pH 7.0 and containing 1% pectin, before and after filtration. The different values obtained after filtration indicate that only a small part of the pectin has been withheld by the candle. This is probably due to slow clogging of the pores of the candle.

With such media and suspension of human and dog feces, representing a normal diet and one to which pectin was added, as sources of bacteria, experiments were devised to study decomposition.

---

[4] McCoy, N., and Peterson, W. H., *J. Inf. Diseases,* 1928, **43**, 475.

TABLE I.

Berkefeld filtration	Phloroglucide formation in g	Viscosity in time	(H)+ Potentiometer
Before	.250	3′ 33″	6.95
After	.220	3′ 5″	6.90

Possible decomposition was followed by physical and chemical examination of the inoculated media after incubation at designated intervals. Results were obtained by observation of gas formation, and determination of titratable acidity, pectate formation, viscosity and hydrogen ion concentration.

Gas production is illustrated by the following typical experiment: This made use of a sterile pectin-broth medium of pH 7.0 and containing 0.5% pectin, which was introduced in equal quantities in fermentation tubes. These were inoculated with a broth suspension of human and dog feces respectively, representing a diet which included pectin. After incubation for 48 hours, the average increase of gas formation in the pectin-broth tubes over the broth control tubes, in the case of dog feces (3 subjects) was 25%, and in the human (3 subjects), 13%. Statistical analysis of the data indicated a significant difference, and it was concluded that the source of the excess gas is from pectin decomposition.

Table II represents experiments in which feces from 4 dogs were used. Nos. 3 and 4 were on a daily ration of one can of commercial dog food (Pard) and 200 cc of milk, and Nos. 1 and 2 on the same diet to which 20 g of pectin were added daily. Inoculations were here made in Erlenmeyer flasks, containing either the pectin-broth medium or the plain broth control medium, diluted with a volume of buffer equal to that used in the pectin medium. The incubation temperature was 37°C and the periods were 24, 48, and 72 hours. Results were obtained by determination of titratable acidity, pectate formation, viscosity and pH. Titratable acidity was carried out with 0.1 N NaOH and phenolphthalein as indicator; pectate formation by the calcium pectate method;[5] viscosity by observing the time necessary to pass through a 10 cc pipette, to which a capillary tube had been fused; pH with a Coleman potentiometer.

Increase in titratable acidity and lower pH values indicate acid production, reduction in time, as a measure for viscosity, proves change in the physical character of the solution, while absence of pectate formation points to a breakdown beyond the gel stage. These changes may be observed by following the data in Table II. It

---

[5] Joseph, Glenn H., Corona, California, personal communication.

TABLE II.

		(H)+ Potentiometer			Titratable acidity cc of 0.1N NaOH			Viscosity in time			Pectate in g		
		Control Before Incubation.											
Pectin*		7.2			2.0			1′ 30″			.82		
Broth†		7.4			1.0			32″			.00		
		Control After Incubation for 72 hr.											
Pectin		7.2			2.0			1′ 32″			.83		
Broth		7.5			1.0			33″			.00		
		Fermentation Periods in hr.											
Media	Dog	24	48	72	24	48	72	24	48	72	24	48	72
		Inoculated and Incubated.											
Pectin	1	6.5	6.0	6.3	2.5	4.5	4.0	1′ 18″	33″	34″	.78	.15	.00
Broth	1	7.2	7.45	7.9	1.0	0.5	0.3	33″	32″	32″	.00	.00	.00
Pectin	2	6.7	5.9	6.3	2.4	5.0	3.8	47″	32″	33″	.67	.12	.00
Broth	2	7.3	7.35	7.5	1.0	0.8	0.7	34″	31″	31″	.00	.00	.00
Pectin	3	6.9	6.6	6.5	2.0	2.5	3.5	1′ 06″	35″	32″	.81	.20	.00
Broth	3	7.25	7.35	8.1	1.0	1.0	0.5	33″	31″	32″	.00	.00	.00
Pectin	4	6.95	6.0	6.6	1.8	5.0	3.3	1′ 16″	32″	33″	.76	.24	.00
Broth	4	7.3	7.4	7.55	1.0	0.7	0.6	33″	31″	32″	.00	.00	.00

*Pectin-broth.
†Broth control.

should be noted that, in 3 of the 4 tests cited in the table, after 48 hours base was produced, which probably arose from the broth.

Work dealing with the isolation of responsible bacteria, determination of the exogenous or endogenous source of the enzyme, and the products of decomposition is being carried out.

Conclusion. The results show that pectin is decomposed by bacteria present in feces obtained from human and canine subjects on a normal diet and one after pectin had been added.

12067 P

Influence of Emotional Excitement on Insulin Content of Blood in Normals and Psychotic Patients.

E. GELLHORN, A. ALLEN AND J. FELDMAN.

*From the Departments of Physiology and Psychiatry, University of Illinois College of Medicine, Chicago, Ill.**

It was shown recently[1] that emotional excitement leads to a dis-

---

* Aided by a grant from the John and Mary R. Markle Foundation and W.P.A. Project 30278.

[1] Gellhorn, E., Cortell, R., and Feldman, J., *Science*, 1940, **92**, 288.

charge over both vago-insulin and sympathetico-adrenal systems, the effect on the latter predominating in the normal animal. These observations are supplemented by the present studies in which the insulin concentration of the human blood was determined by an assay on the hypophysectomized-adreno-demedullated rat.[2]

If 1 cc of normal human blood is injected intraperitoneally into such rats and the blood sugar is determined after one hour a very slight fall in blood sugar is observed and the animals do not show any symptoms (A). If, however, excited psychotic patients are tested it is found that their blood produces a marked hypoglycemia in test animals, frequently leading to coma or hypoglycemic convulsions. It is noteworthy that this result was regularly obtained with the blood of 13 excited patients (C, Table I) whose blood sugar was unaltered. Moreover, in 2 cases (G) it could be shown that in quiet periods following the excitatory state the test no longer indicated increased amounts of insulin in the blood but produced a reaction similar to that of our control cases. Apparently emotional excitement leads in these cases predominantly to an increased secretion of insulin, whereas in normals the effect on the sympathetico-adrenal system prevails.[1, 3] This conclusion is supported by the comparative study of Groups B, C, and D. Students excited by the writing of the semester examination served as controls. Their blood sugar was somewhat elevated and caused a slight rise in the blood sugar of the test rat (B). The blood of an excited patient with a similar hyperglycemia (D) caused a conspicuous hypoglycemia in the test animal. Even in the case of Group E in which emotional excitement led to a more marked hyperglycemia indicating a much greater adrenalin secretion than in the excited controls (B), the test animal reacted

TABLE I.
Emotional Excitement and Insulin Content of Human Blood.

Group	No.	Blood sugar of the subject	Test	Remarks
A	7	78.9	57.4*-51.4†	Normals, quiet
B	5	96.5	58.4 -61.5	,, excited
C	13	78.8	59.0 -34.4	Psychotics, excited
D	1	94.6	60.2 -39.7	,, ,,
E	3	106.2	58.7 -51.2	,, ,,
F	2	80.3	58.0 -34.9	,, ,,
G	2	75.2	59.1 -52.1	Same patients, quiet as in F

* Blood sugar of the hypophysectomized-adreno-demedullated rat before injection of 1 cc blood/100 cc rat.

†Blood sugar of test rat one hour after injection.

---

[2] Allen, A., Feldman, J., and Gellhorn, E., *Proc. Am. Physiol. Soc.*, April, 1941.

[3] Gellhorn, E., Allen, A., Cortell, R., and Feldman, J., *Proc. Am. Physiol. Soc.*, April, 1941.

with a slight hypoglycemia similar to that found in the unexcited control group. Obviously, the insulin concentration of the blood was increased sufficiently to offset completely the effects of adrenalin on the blood sugar of the test animal.

With respect to the reactivity of the vago-insulin and the sympathetico-adrenal systems our experiments can be divided into 3 groups. The first consists of excited controls showing sympathetico-adrenal effects but no signs of vago-insulin excitation; the second group consists of the majority of psychotic patients showing absence of sympathetico-adrenal effects (blood sugar unchanged) and greatly increased insulin content of the blood. Finally, the third group comprises excited patients in a somewhat intermediate group in which the reactivity of the sympathetico-adrenal system is adequate to cause a rise in the blood sugar of the patient but the test on the hypophysectomized-adreno-demedullated rat reveals that insulin is present in increased amounts enough to offset the effect of adrenalin on the blood sugar of the test animal.

12068 P

Heart Sounds in Myocardial Infarction Due to Acute Coronary Occlusion.

RUDOLPH FRIEDMAN AND ARTHUR M. MASTER.
From the Cardiographic Laboratory, Mount Sinai Hospital, New York City.

In 80 cases of acute coronary occlusion a phonocardiographic study was made of the effect of myocardial infarction on the character of the heart sounds. Using the Sanborn Stetho-cardiette, phonocardiograms were recorded daily during the first 2 or 3 weeks following the attack and 3 times weekly thereafter until discharge. The apical heart sounds were recorded with the patient in the semi-recumbent position and holding his breath in expiration. For proper timing, an electrocardiogram and venous pulse curve were recorded simultaneously.

The first and second heart sounds were analyzed for intensity (amplitude), pitch (frequency) and duration, and the presence of normal or accentuated auricular and third heart sounds was noted. The analysis was aided by the use of 2 microphones, one amplifying all sound frequencies equally, including those of very low pitch which are usually inaudible, and the other selectively amplifying the

TABLE I.
The Heart Sounds in Acute Coronary Occlusion: Comparison with Normal Subjects of the Same Age Group.

	Coronary occlusion	%	Normal subjects
No. of cases	78		100
First sound			
S_1 low	19	(33)	6
S_1 lower than S_2	41	(57)	6
Auricular sound			
Total No.	65	(83)	38
Normal	38	(50)	38
Accentuated (presystolic gallop)	26	(33)	0
Third sound			
Total No.	37	(47)	12
Normal	30	(38)	12
Accentuated (protodiastolic gallop)	7	(8)	0
Summation gallop	5	(6)	0

sounds of higher frequency, thus corresponding to auscultation by the human ear. For comparison, phonocardiograms were also recorded in a control series of 100 normal subjects, 93% of whom were over 40 years of age.

The results are tabulated in Table I. The significant findings are related to the first sound, the auricular and the third sounds.

1. *First Heart Sound.* a. *Intensity:* A distinct diminution of the amplitude of this sound occurred in 33% of cases. In 57% the first sound showed a relative diminution as compared to the second. This is the reverse of the normal relationship, being present in only 6% of the normal control group. The diminished first sound in normal individuals is related to various extra cardiac factors affecting sound conduction, such as thickness of the chest well, form of the thoracic cage and overlying lung tissue.

b. Frequency: The diminution in amplitude involved exclusively the high frequency component, resulting in a first sound of low pitch and decreased audibility. The main high frequency component of the normal first sound originates during the isometric phase of ventricular systole. Its disappearance following myocardial infarction is probably related to lowered intraventricular pressure and diminished force of ventricular contraction.

c. Duration: This was not affected in any of the cases, unless intraventricular block was present.

2. *Auricular Sound.* An auricular sound immediately preceding the first sound and which is related to auricular contraction, occurred in 83% of cases of infarction as compared to 38% of the normal control group. It was of normal amplitude in 50% and distinctly accentuated in 33%. (It was never accentuated in the normal group.)

The accentuated sound usually became audible as a presystolic gallop rhythm. It is related to the increased intraäuricular pressure following ventricular infarction and heart failure.

3. *Third Heart Sound.* A third sound, occurring in early diastole, was present in 47% of cases of infarction and in only 12% of normal subjects. It was definitely accentuated in 8%, forming an audible protodiastolic gallop rhythm. The third sound is related to the early diastolic filling of the ventricles when the inrushing blood from the auricles impinges on the ventricular wall. A decreased ventricular tonus and increased intraäuricular pressure which occur in infarction favor the production of a third sound of increased intensity.

In 6% of the cases there was a superimposition of the normal or accentuated auricular and third sound forming a clinically audible summation gallop rhythm.

Summary. In myocardial infarction due to acute coronary occlusion the first sound is usually diminished in amplitude both absolutely and relatively to the second sound, and is of low pitch owing to the small amplitude of the high frequency component. There is an increased incidence of auricular and third sounds. The accentuation of these sounds by increased intraäuricular pressure and decreased ventricular tonus often results in gallop rhythm.

12069

Liver Histamine During Peptone Shock in Dogs.

CARL A. HOLMES, GAYLORD OJERS AND CARL A. DRAGSTEDT.
From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Dragstedt and Mead[1] reported that the vascular shock produced by the intravenous injection of proteose solutions into dogs is accompanied by the appearance in the blood and thoracic duct lymph of a vasodepressor substance which was tentatively identified as histamine. Dragstedt, Mead and Eyer[2] presented additional evidence that the vasodepressor substance was histamine. They likewise presented indirect evidence that the amount of histamine liberated from the fixed tissues is adequate to account for the degree of shock and concluded that "the vascular shock produced by the intravenous injection of peptone solutions (which contain proteose) into dogs is due to the liberation of histamine from the tissues into the circulating blood." Feldberg and O'Connor[3] have reported that perfusing guinea pigs' lungs with peptone solution leads to the appearance of histamine in the perfusate and have concluded that peptone causes a type of cell injury with resultant liberation of histamine. Their observations on guinea pig lungs have been confirmed in this laboratory.

---

[1] Dragstedt and Mead, *J. Pharm. and Exp. Ther.*, 1937, **59**, 429.

[2] Dragstedt, Mead and Eyer, *J. Pharm. and Exp. Ther.*, 1938, **63**, 400.

There is a certain amount of circumstantial evidence indicating that the liver is the major tissue concerned in the reaction in the dog. For example, the coagulation defect in peptone shock is commonly considered as an hepatic reaction.[4] The experiments here reported were planned as a test of the histamine liberation theory, and the liver was chosen as the tissue to be studied. Biopsy specimens of liver tissue were obtained before and after the injection of peptone solutions, analyzed for their histamine content, and the changes in liver histamine correlated with the degree of the reaction produced as indicated by the blood pressure responses.

Method. Dogs were used. All animals were anesthetized with ether or sodium barbital, and records made of the carotid pressure. A 10% solution of Bacto-Protone (Difco) was used; all solutions were freshly prepared, acidified, shaken with permutit, and then filtered and neutralized prior to use. This procedure removes more or less completely the vasodepressor constituents of the solution so that the blood pressure fall obtained measures the degree of the "peptone shock" without any confusion from the direct effect obtained from many peptone solutions. A dose of 2 cc per kilo was injected rapidly via the femoral vein.

It has been noted previously[1] that there is no direct correlation between the dose of peptone injected and the degree of effect produced. The dose here used (2cc per kilo) has been found to produce reactions in various animals that range from insignificant changes in the blood pressure to a rapidly fatal shock. Consequently, while it is not necessary to vary the dose of peptone in order to obtain reactions of varying degrees for study, it is obviously important to determine in each experiment the degree of the reaction experienced. The degree of the resulting reaction was determined from the blood pressure record and expressed by the following arbitrary schema: ++++ = shock fatal within 30 minutes, +++ = severe shock with blood pressure still at shock level 30 minutes after injection, ++A = shock with blood pressure at shock level for 20 minutes but with some recovery before 30 minutes, ++B = shock with blood pressure at shock level for 10 minutes but with some recovery before 20 minutes, ++C = shock with blood pressure falling to shock level but with recovery before 10 minutes, + = mild reaction with a slight temporary fall of blood pressure. The abdomen was opened, and just prior to and again a few minutes after the injection of peptone a 2 to 4 g portion of liver was excised. Care was taken to secure hemostasis. These liver samples were analyzed for their histamine content by the method of Best.[5] The assays were made on an etherized, atropinized cat against known dilutions of histamine acid phosphate. At the conclusion of the experiment the dog's liver was removed and weighed, after permitting the freely draining blood to escape. Calculations of the total liver histamine were made on the assumption that there is an even distribution of histamine throughout the liver. Several control experiments indicate that this

---

[3] Feldberg and O'Connor, *J. Physiol.*, 1937, **90**, 288.

[4] Wöhlisch, *Ergebn. Physiol.*, 1929, **28**, 443.

TABLE I.
Liver Histamine During Peptone Shock in Dogs.

1	2	3	4	5	6	7	8	9	10
1	13.4	394	+	37.5	15.0	50.0	19.7	—	—
2	10.0	445	+	75.0	33.4	75.0	33.4	—	—
3	10.7	252	+	35.0	8.8	35.0	8.8	—	—
4	6.8	332	+	150.0	49.8	150.0	49.8	—	—
5	9.3	305	++C	120.0	36.6	120.0	36.6	—	—
6	8.0	230	++C	75.0	17.2	48.0	11.0	6.2	0.77
7	10.0	260	++C	150.0	39.0	105.0	27.3	11.7	1.1
8	15.2	580	++B	150.0	87.0	105.0	61.0	26.0	1.7
9	5.5	175	++A	150.0	26.0	67.0	12.0	14.0	2.5
10	6.1	223	+++	150.0	33.5	37.5	8.4	25.1	4.1
11	6.1	287	+++	135.0	38.0	60.0	17.0	21.0	3.5
12	6.4	270	+++	170.0	46.0	75.0	20.0	26.0	4.0
13	8.3	378	+++	135.0	51.0	50.0	19.0	32.0	3.8
14	10.7	441	++++	105.0	46.3	75.0	33.0	13.3	1.2
15	6.4	273	++++	165.0	45.0	52.5	14.0	31.0	4.8

Column 1—Exp. No.
,, 2—Wt of dog (kg).
,, 3—Wt of liver (g).
,, 4—Degree of shock—see text.
,, 5—Pre-shock liver histamine equivalent in μg of histamine acid phosphate per g.
,, 6—Total pre-shock histamine equivalent in mg of histamine acid phosphate.
,, 7—Post-shock liver histamine equivalent in μg histamine acid phosphate per g.
,, 8—Total post-shock liver histamine equivalent in mg of histamine acid phosphate.
,, 9—Total amt of histamine liberated from liver in mg.
,, 10— ,, ,, ,, liberated histamine in mg per kilo of dog.

---

[5] Best, *J. Physiol.*, 1929, **67**, 256.

is a valid assumption. The results are shown in Table I, in which, for the sake of convenience, the experiments have been listed in the order of increasing severity of the shock reactions obtained.

Discussion. It will be noted that in every instance of a severe reaction there was a marked reduction in the liver histamine, while there was no reduction in the animals that had mild reactions. In one of these (Exp. 1) there appeared to be an increase in the liver histamine. Whether this is a real increase or merely an assay error is not possible to say. Tarras-Wahlberg[6] has reported that various procedures such as asphyxia result in an increase in the lung histamine of cats and rabbits. While his findings cannot properly be applied to the present experiments, they show that various non-shocking experimental procedures tend to result in an increase rather than a decrease in the tissue histamine. In the present experiments, the substantial reductions in liver histamine, amounting in 6 instances to more than 50% of the initial value, are considerably beyond any assay error. The histamine lost by the liver, calculated in terms of the body weight of the animal (Column 10 in the table), ranges from 0.77 to 4.8 mg of histamine acid phosphate per kilo. With the single exception of Experiment 14, the degree of the shock reaction is extremely well correlated with the amount of histamine lost by the liver; and even in this experiment the reaction is not beyond what can be accounted for by a corresponding dose of histamine.[7] It is highly probable that proteoses lead to the liberation of histamine from other tissues than the liver, but the results here reported would indicate that such other sources are relatively insignificant in the pathogenesis of the systemic reaction since the quantity liberated by the liver is substantially adequate to account for the degree of shock in every case. For the same reason it can be stated that tissue constituents other than histamine, if they are concerned at all, would appear to be of minor significance in the reaction. This statement applies, of course, only to the vascular reaction. The coagulation defect in peptone shock has been demonstrated to be due to the liberation of heparin.[8, 9]

Conclusion. Peptone shock in the dog is accompanied by a reduction in the liver histamine which is roughly parallel to the degree of the shock reaction. The amount of histamine liberated from the liver is substantially adequate to account for the degree of shock as determined by the systemic blood pressure.

---

[6] Tarras-Wahlberg, *Skand. Arch. f. Physiol.*, 1936, **73**, Supp. 1.

[7] Dragstedt and Mead, *J. Pharm. and Exp. Ther.*, 1936, **57**, 419.

[8] Quick, *Am. J. Physiol.*, 1936, **116**, 535.

[9] Waters, Markowitz and Jacques, *Science*, 1938, **87**, 582.

12070

Hysterectomy at Parturition and Ovarian Function in the Monkey (*M. mulatta*).

G. van Wagenen* and H. R. Catchpole.

From the Department of Obstetrics and Gynecology and the Adolescence Study Unit, Yale University School of Medicine, New Haven, Conn.

Whether the endometrium responds only as an end organ, or whether it affects or modifies the hypophyseal-ovarian relationship remains an open question. While much clinical evidence points to an active rôle, the experiments of Burford and Diddle[1] on hysterectomy in immature and adult monkeys tend to show that the operation in no way modified ovarian physiology as demonstrated by signs of continued ovulation, changes in the sex skin and vagina, and by attainment of puberty.

We have attempted to obtain evidence on this question by observing the course of recovery of ovarian activity following parturition in normal monkeys and in monkeys deprived of their uteri at the time of parturition. During the latter part of pregnancy, and at term, the ovary of the macaque is apparently functionally quiescent. The ovaries become translucent in appearance and are elongated or spindle-shaped. There is an absolute reduction in their size and weight. Corpora lutea, large and medium follicles are absent and macroscopically the ovary presents a picture of regression and suppression. It was felt that the process by which such a gonad is again brought to full cyclical activity might be peculiarly susceptible to the absence of the uterine endometrium, if the latter is implicated in any important way in this process.

Experimental. Five normal pregnant monkeys were delivered at term and their babies were immediately separated from the mothers. Two of the animals on the 4th and 6th postpartum day, respectively, were subjected to a supravaginal hysterectomy whereby all but an insignificant amount of the cervical mucosa was removed.

As a measure of ovarian function we determined thrice weekly the percentage sedimentation in the vaginal lavage, according to the technic of Hartman.[2] Desquamation and menstrual cycles in the 3 control animals and desquamation cycles in the 2 experimental

---

* Aided by funds from the John and Mary R. Markle Foundation.

[1] Burford and Diddle, *Surg., Gynec. and Obst.*, 1936, **62**, 701.

[2] Hartman, Carl G., *Contrib. to Embryol.*, Carnegie Institution of Washington, 1932, Vol. 23, p. 1.

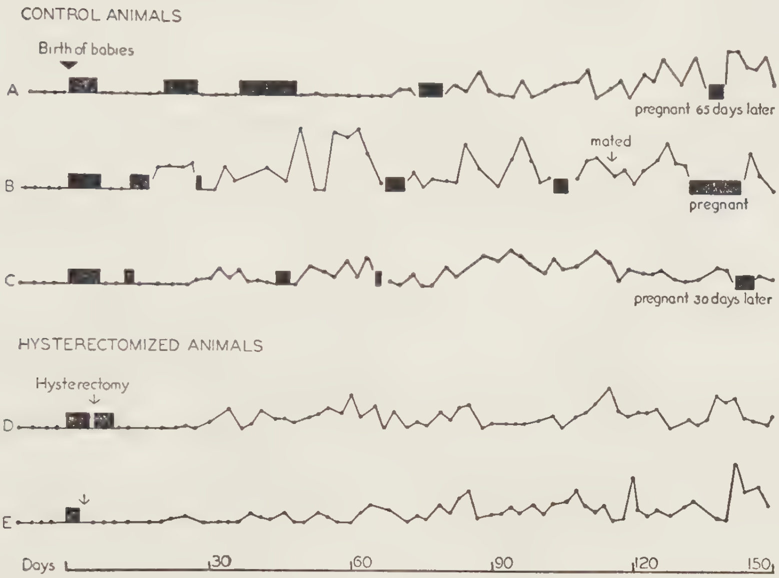


FIG. 1.

Curves showing vaginal desquamation in 5 monkeys followed for 150 days after parturition. Two of the animals were hysterectomized on the 6th and 4th days after delivery. The solid areas represent vaginal bleeding. In animal B the last bleeding shown is the placental sign indicating a positive mating at the 115th day.

animals are shown in Fig. 1. In the normal animals (A, B, and C), the first signs of desquamation were observed at 70, 19, and 27 days, respectively, after parturition; corresponding maximum sedimentation values were 50, 65, and 40%. In monkey A there occurred uterine bleeding at intervals following the lochial discharge which was associated with the persistence of a gestational type of vaginal epithelium. First menstrual bleeding occurred 75 days after delivery and an appreciable sediment reappeared in the lavage at this time. In monkey B a cycle of relatively normal length was established early, suggesting that the animal might be mated, and pregnancy immediately ensued. In the animals D and E from which the uterus had been removed there was, of course, no menstruation, but they resumed vaginal desquamation at 30 and 20 days, respectively, after parturition and showed maximum sedimentation values of 45% and 60%. The sedimentation curves for the 5 animals are, in fact, virtually indistinguishable. The 3 controls were mated and became pregnant at 215, 105, and 180 days respectively, after the birth of their last babies. Thus, their desquamation curves may be accepted as typical records of normal, cyclic animals capable of reproduction and the curves for the experimental animals were not shown to deviate from them to any degree.

Conclusion. Supravaginal hysterectomy in the macaque at the time of parturition causes no deviation from the normal pattern in the resumption of ovarian activity as demonstrated by the study of vaginal desquamation.

12071

Detoxication of Borneol by Glucuronic Acid in Humans.

HARRY WAGREICH, ABRAHAM BERNSTEIN, MORTON PADER AND BENJAMIN HARROW.

*From the Department of Chemistry, The City College, College of the City of New York.**

In previous studies[1] we had investigated the extent of detoxication of phenylacetic acid and benzoic acid in the form of glucuronides, using the photoelectric colorimetric method of Maughan, Evelyn and Browne.[2] We were attracted to the study of the detoxication of borneol for the reason that the high percentage of detoxication by glucuronic acid of this substance reported in the literature enhanced the possibility of using it to study pathological cases. In this paper we deal with a detailed study of normal cases.

Quick[3] reporting 2 cases in which 2 g of borneol was ingested by human subjects, stated that 81% was excreted as the glucuronide within 10 hours after ingestion; while on giving the subject 3.5 g of borneol, 69% was thus excreted within 6 hours. Pryde and Williams[4] extended these experiments to 24 human cases and measured the excretion of glucuronides for some stated period between 6-12 hours after the ingestion of 2 g of borneol. These authors found that the maximum excretion was around 80%, and that this limit is reached in the normal human being within 10-12 hours. It should be added that both Quick, and Pryde and Williams determined their glucuronides by less convenient reduction methods.[5]

---

* We wish to acknowledge the help of Mr. William Lewanda in carrying out some of the analyses.

[1] Wagreich, H., Kamin, H., and Harrow, B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 468; Wagreich, H., Abrams, A., and Harrow, B., *Ibid.*, 1940, **45**, 46.

[2] Maughan, G. B., Evelyn, K. A., and Browne, J. S. L., *J. Biol. Chem.*, 1938, **126**, 567.

[3] Quick, A. J., *J. Biol. Chem.*, 1928, **80**, 535.

[4] Pryde, J., and Williams, R. T., *Biochem. J.*, 1936, **30**, 799.

In their paper, Pryde and Williams fail to present analyses of urinary glucuronides for each subject *before* the ingestion of the borneol and *after* the 12-hour period following the ingestion. In order to determine the maximum detoxication, the urine from each subject was collected for a given period (from 6-12 hours), and the urinary glucuronides were determined in the total volume of urine voided. For each subject, therefore, with one exception, only *one* figure is given by Pryde and Williams for the excretion of the glucuronides.

We report, first, the results of 8 subjects (male students, ranging in age from 17-22 years) when fed 2 g of borneol in gelatin capsules. Twenty-four-hour urine samples were collected for 2 days. At the beginning of the third day, the subject ingested 2 g of borneol. The collection of urine was then continued for 3 days.

The average percentage detoxication for these cases with 94% (the range was 79 to 104%), which is somewhat higher than that obtained by Pryde and Williams.

The figures for the fourth and fifth days show that the urinary glucuronides tend to return to the normal range one day after ingestion of the borneol.

Preliminary work with pathological cases convinced us that 2 g of borneol was an inconvenient dose to ingest. We therefore turned to a detailed study of the ingestion of 1 g of borneol, using normal subjects throughout. The experimental procedure was similar to that adopted for 2 g of borneol with this modification: that on the

TABLE I.
Amount of Glucuronic Acid Eliminated on Ingestion of 2 g of Borneol.

Subject	Mg glucuronic acid per 24-hr sample days					Avg mg glucuronic acid per 24 hrs in normal urines†	Increase in mg glucuronic acid on ingestion of 2 g borneol	% detoxication
	1	2	3*	4	5			
A	432	343	2889	455	431	388	2501	99
B	551	497	2993	664	475	524	2469	98
C	418	543	2814	546	348	481	2333	93
D	623	516	3182	541	702	570	2612	104
E	355	572	2890	723	687	464	2426	96
F	477	517	2487	685	559	497	1990	79
G	578	452	2866	557	388	515	2351	93
H	649	629	3099	412	634	639	2460	98

*Ingestion of borneol at the beginning of the third day.
†These figures were obtained by taking the average of the first 2 days.

---

[5] Quick, A. J., *Ind. Eng. Chem.*, 1925, **17**, 729.

third day—the day of ingestion—the subject collected *5 three-hour samples of urine,* followed by 1 nine-hour sample to complete the collection for that day.

Table II presents the results using 26 normal subjects. Here the results are tabulated in 24-hour intervals. The percent detoxication on the ingestion of 1 g of borneol varies from 60 to 101, with an average of 81. Again, as with the 2 g experiments, the urinary glucuronides return to normal within one day after feeding.

Table III gives the results of the elimination of glucuronides at each 3-hour interval, and the 9-hour interval on the day the subject was fed 1 g of borneol.

Fifty percent of the cases showed a maximum percent detoxication in the second 3-hour interval, while 35% reached a maximum in the third 3-hour period. The amount of glucuronic acid obtained during that 3-hour period in which maximum elimination takes place varies from 412-897 mg with an average of 605 mg.

TABLE II.
Amount of Glucuronic Acid Eliminated on Ingestion of 1 g Borneol.

Subject	Mg glucuronic acid per 24-hr sample, days					Avg mg glucuronic acid per 24 hrs in normal urines†	Increase in mg glucuronic acid on ingestion of 1 g borneol	% detoxication
	1	2	3*	4	5			
1	350	569	1379	338	384	460	919	73
2	366	515	1671	542	510	441	1230	98
3	435	738	1710	711	350	587	1123	89
4	356	509	1557	406	399	433	1124	89
5	546	559	1912	512	487	553	1359	108(?)
6	583	466	1434	550	764	525	909	72
7	603	552	1393	563	814	578	815	65
8	575	528	1698	482	552	552	1146	91
9	318	425	1549	538	475	372	1177	93
10	612	562	1351	558	515	587	764	61
11	577	542	1456	322	560	560	896	71
12	382	398	1192	430	389	390	802	64
13	420	402	1410	394	442	411	999	80
14	326	477	1256	400	411	402	854	68
15	592	424	1282	638	590	508	774	62
16	322	396	1613	455	445	359	1254	100
17	459	384	1391	482	322	422	969	76
18	523	444	1622	680	575	484	1138	90
19	396	492	1398	521	689	444	954	76
20	539	705	1805	645	430	622	1183	94
21	490	410	1722	383	372	450	1272	101
22	585	510	1299	662	600	548	751	60
23	683	970	1728	695	774	827	901	72
24	563	739	1638	608	572	651	987	79
25	397	382	1555	531	304	390	1165	93
26	398	576	1575	572	564	487	1088	87

*Ingestion of borneol at the beginning of the third day.
†These figures were obtained by taking the average of the first 2 days.

TABLE III.
Amounts of Glucuronic Acid in mg Eliminated in Stated Intervals after Ingestion of 1 g of Borneol.

Subject	3-hr intervals First	 Second	 Third	 Fourth	 Fifth	9-hr interval Sixth	Hours during which max. production of glucuronides occurred
1	52	46	223	450	320	288	9-12
2	105	313	729	260	74	190	6- 9
3	300	816	138	—*	199	257	3- 6
4	167	585	351	135	80	191	3- 6
5	187	147	824	281	235	238	6- 9
6	335	329	152	139	189	290	0- 6
7	200	538	208	275	83	89	3- 6
8	183	736	314	120	172	175	3- 6
9	82	597	329	205	110	226	3- 6
10	130	455	221	110	105	330	3- 6
11	73	146	677	175	131	254	6- 9
12	50	64	148	539	194	297	9-12
13	79	779	169	146	86	151	3- 6
14	66	224	412	159	139	256	6- 9
15	173	480	200	103	98	227	3- 6
16	146	306	231	534	146	250	9-12
17	140	52	781	140	98	180	6- 9
18	67	509	367	187	160	332	3- 6
19	112	287	417	156	165	261	6- 9
20	180	381	663	211	97	273	6- 9
21	64	206	897	276	98	181	6- 9
22	256	503	154	117	90	179	3- 6
23	237	590	195	189	195	322	3- 6
24	281	500	282	173	164	238	3- 6
25	194	552	266	145	157	241	3- 6
26	88	395	550	212	195	135	6- 9

*Subject did not void any urine during this period.

The average percentage detoxication which occurs within 15 hours after the ingestion of 1 g of borneol is 78 (the range being from 58 to 100).† Since the average percentage detoxication for a 24-hour period is 81, this indicates that detoxication is almost complete within 15 hours after the ingestion of the borneol.

Summary. (1) The ingestion of 2 g of borneol by 8 normal subjects resulted in the excretion, within 24 hours, of an average of 94% in the form of its glucuronide, the range being from 79 to 104%. (2) The ingestion of 1 g of borneol by 26 normal subjects resulted in the excretion, within 24 hours, of an average of 81%, the range being from 60 to 100%. (3) Fifty percent of the subjects, on ingesting 1 g of borneol, gave a maximum excretion in 3 to 6 hours, while 35% gave it in 6 to 9 hours. (4) The detoxication of 1 g of borneol by the 26 subjects was almost complete in 15 hours, the average value at that time being about 96% of that obtained in 24 hours.

---

† These values were obtained by subtracting $^{15}/_{24}$ times the average of the daily normals from the values of glucuronic acid obtained by adding the 5 three-hour periods shown in Table III. This value was then divided by the theoretical value, in mg, for 100% detoxication.

12072

Influence of Lactogenic Preparations on Production of Traumatic Placentoma in the Rat.*

Herbert M. Evans, Miriam E. Simpson and William R. Lyons.

From the Institute of Experimental Biology and the Department of Anatomy, University of California, Berkeley.

For several years we have been concerned with the problem of the hormonal factors involved in the establishment and maintenance of pregnancy, hoping ultimately to understand the pituitary factors necessary for nidation and maintenance of pregnancy in the hypophysectomized rat. For the present we have had to limit ourselves to an examination of the factors necessary for maintenance of pregnancy in the precociously matured rat and to an examination of the pituitary factors necessary for the production of *functional* corpora lutea, as judged by the ability to produce deciduomatous tissue in the traumatized endometrium. These studies on the effect of various fractions of the pituitary on placentoma formation will be reported elsewhere[1] *in extenso,* the present paper being limited to the effect of lactogenic preparations.

Evans and Long showed many years ago that the corpora lutea of the normal cyclic rat were non-functional and would not sustain placentomata unless the cycle was prolonged by stimulation of the cervix. They further showed that crude pituitary extracts would also prolong the cycle, and Teel showed that traumatic placentomata could be produced under the latter conditions. It is relatively easy to produce corpora lutea in the ovaries of immature rodents or of hypophysectomized rats with gonadotrophic agents but such corpora lutea like normally occurring cyclic corpora are usually non-functional as judged by the placentoma test.

---

* Aided by grants from the Research Board of the University of California and from the Rockefeller Foundation. Assistance was rendered by the Works Projects Administration, Official Project No. 65-1-08-62., Unit A-5.

[1] Evans, H. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K., *Endocrinology,* in press.

Among all the purified pituitary fractions investigated in the last few years by the placentoma test, we have found that the lactogenic hormone is the only one which will lead to functional corpora lutea in hypophysectomized rats. In the earlier report[2] on this subject some doubt remained whether adrenocorticotrophic hormone contaminating the lactogenic preparation might be the effective factor. Since that time lactogenic hormone has been further purified and it elicits no adrenal response even when given in extremely high doses (50 mg doses in 10 days in hypophysectomized rats) so that this doubt has been minimized. It is true that several pituitary factors will, in high doses, and somewhat sporadically, lead to placentoma formation in normal rats, but this effect is probably indirect, through stimulation of the pituitary to secrete lactogenic hormone.

The placentoma test as utilized here is as follows: young mature female rats about 65 days of age, possessing newly-formed lutein tissue, are injected for 5 days with the pituitary substance under investigation. Threads are then inserted in the uterus on the day of the fifth injection, care being taken that the thread actually passes through the endometrium. Injections are continued for the next 5 days and the animals are sacrificed on day 11 of the experiment. In the case of hypophysectomized animals, injections are begun on the day of operation and the procedure is otherwise the same. Under the proper conditions discrete enlargements of the uterus occur at the point where the endometrium is injured. In the 6-day period of growth the enlargements attain a diameter of 0.5-0.6 cm. Microscopically they can be seen to be composed of tissue resembling the maternal part of the placenta.

Table I shows the reaction of hypophysectomized animals injected with lactogenic hormone containing approximately 30 International Units per mg. It will be noted that at the 1 mg level all animals

TABLE I.
Stimulation of Placentomata in Hypophysectomized Rats on Administration of Lactogenic Hormone.
Rats 65 Days Old at Hypophysectomy.

Total dose in 10 days		No. of rats	
mg	IU	Total	Positive
50	1500	4	4
10	300	4	4
5	150	4	2
0	0	13	0

---

[2] Evans, H. M., Simpson, M. E., and Turpeinen, K., *Anat. Rec.*, Suppl. 3, 1938, **70**, 26.

responded; at 0.5 mg daily dosage approximately 50% were positive. The controls receiving no lactogenic hormone uniformly failed to respond to threads passed through the endometrium.

Immature animals, therefore smaller in body weight, in which corpora lutea had been artificially produced by injection of gonadotrophin (29-day-old rats injected subcutaneously with pituitary FSH, 15 RU total dose in 3 days) also formed placentomata around threads, if this treatment was followed by lactogenic hormone. Six rats given 0.5 mg of lactogenic hormone daily and 6 given 0.1 mg daily all showed placentomata, indicating that 0.1 mg was above the MED in this age group. None of the 6 controls, whose ovaries were luteinized, and in which threads were inserted, but which received no lactogenic hormone, responded by placentoma formation. As may be seen in Table II, adult hypophysectomized rats in which corpora lutea were induced by a gonadotrophic injection, also failed to nidate threads if not given supplementary therapy.

The corpora lutea present at hypophysectomy survive for a long period judging by histological appearance. They can, however, only be brought to functional condition by lactogenic hormone if injections are begun immediately after hypophysectomy. Table II summarizes one experiment which illustrates this point. When injections were begun immediately after operation, 1 mg daily doses were effective in 100% of the cases. When a period of 10 days or more—13 in this case—was allowed to elapse between operation and the onset of injection, no placentomata resulted. However, as the lower part of the table shows, when a new crop of corpora lutea was produced a considerable period after operation by the injection of gonadotrophic agents (in this case pituitary FSH (10 RU) and human chorionic gonadotrophin (1 RU) in 3 daily subcutaneous injections), and this priming was followed by lactogenic hormone,

TABLE II.
Influence of Post-operative Period on Stimulation of Placentomata in Hypophysectomized Rats by Lactogenic Hormone.
Rats 65 Days Old at Hypophysectomy.

Post-operative period in days				Total dose in 10 days		No. of rats	
Onset of injection	Threads inserted	Autopsy	No. of days injected	mg	IU	Total	Positive
0	4*	10	10	10	300	4	4
13	17	23	10	10	300	3	0
Corpus luteum formation induced by pituitary FSH and chorionic gonadotrophin, days 10 to 12 post-operative.							
13	17	23	10	10	300	3	3
—	17	23	—	—	—	7	0

*Day of 5th injection.

the newly formed corpora could be brought to functional condition and the animals would nidate threads. The last line of the table shows that these newly formed corpora lutea were unable to support placentomata without supplementary lactogenic hormone therapy.

It is possible to distinguish histologically those corpora lutea which are capable of supporting placentomata. They are large and are composed of large cells, in both respects reminding one of the corpora of pregnancy. The sparse, fat-laden interstitial tissue is also similar to that seen in pregnant rats.

It is of interest to note that the dose of lactogenic hormone found effective in producing placentomata (0.1 mg daily in immature rats and 0.5 mg daily in mature rats) is more than the minimal systemic dose necessary to stimulate the pigeon crop (0.02 mg given subcutaneously daily for 4 days) and less than that necessary to initiate lactation in the estrous guinea pig (1 mg given subcutaneously daily for 4 days).

The lactogenic hormone used is the purest pituitary product prepared in this laboratory, and although it is not crystalline, there is perhaps even stronger evidence for its purity. It has been shown to be homogeneous both by the electrophoretic test (Tiselius) and by solubility tests.[3] The assumption is made here, due to the degree of purity of the preparation, that it is the lactogenic hormone itself which is concerned in inducing the functional state of the corpus luteum. It is true that in certain other instances in the past very minute amounts of contamination in relatively purified hormonal preparations have proven to be the active portions of the product, *i. e.,* the sensitiveness of some biological tests equals or exceeds our best physical and chemical tests for purity.

Astwood[4] has recently published a paper also reporting a pituitary fraction (called "luteotrophin") which brings corpora lutea to a functional state. His test is somewhat different than that here described. Astwood's test consists in injecting 23-30-day-old rats with 2 IU chorionic gonadotrophin daily for 4 days. When estrus occurs, he injects 20 gamma estradiol dipropionate, followed by a maintenance dose of 10 gamma of the same material twice weekly. After 1-3 days of vaginal cornification, the vagina shows the mucification reaction indicative of active corpora lutea. The rats are then hypophysectomized and injected with "luteotrophin" twice daily for 4-14 days. If no estrus occurs in that period (that is, vaginal mucification is sustained) he considers the test positive for "luteotrophin."

---

[3] Li, C. H., Lyons, W. R., and Evans, H. M., *J. Gen. Physiol.,* 1940, **23**, 433; *J. Am. Chem. Soc.,* 1940, **62**, 2925; *J. Gen. Physiol.,* 1941, **24**, 303; *J. Biol. Chem.,* in press.

[4] Astwood, E. B., *Endocrinology,* 1941, **28**, 309.

This test is, doubtless, thoroughly satisfactory for measuring lutein function and as far as can be judged, the minimum effective doses for the production of the phenomena described by Astwood and by this laboratory are essentially the same (0.1 mg or less). Although one of Astwood's methods for the preparation of "luteotrophin" is that described by Lyons for lactogenic hormone, he nevertheless has not identified this active factor with the lactogenic hormone, inasmuch as he designates it by a new term. His inability to cause the proliferation of mammary lobules in hypophysectomized rats with some of his luteotrophic preparations apparently forms the basis for his unwillingness to identify this factor with lactogenic hormone. However, as far as we are aware, no one has ever caused proliferation of mammary lobules in hypophysectomized rats with any amount of lactogenic hormone,[†] nor has any one used this as a method of identifying lactogenic hormone.

The series of responses described by Astwood can perhaps be explained and harmonized with the findings and explanations given here as follows. He luteinizes the immature rat ovary by injection of chorionic gonadotrophin. The injection of estradiol dipropionate then stimulates the pituitary to secrete a substance "luteotrophin" (identified by us with lactogenic hormone) which causes the lutein tissue to secrete progestin. This is indicated by vaginal mucification. After hypophysectomy, unless the luteotrophin is injected the lutein tissue no longer functions in maintaining the mucification, and vaginal cornification ensues from the continued injection of estrin. It is our experience that in the normal adult rat, daily injections of 0.5 mg of lactogenic hormone induce vaginal mucification and maintain it for long periods. It is assumed that here too, the lactogenic hormone is effective through stimulation of already existing lutein tissue to secrete progestin.

Summary. Normally occurring or artificially induced lutein tissue in the rat does not produce progestin, as shown by the placentoma test. The only pituitary preparation which has been shown to stimulate the production of progestin by such lutein tissue is lactogenic hormone. Therefore, besides its classical mammotrophic activity and crop-sac stimulating function, the "lactogenic hormone" is important in activation of the corpus luteum, and for this reason should be considered a part of the gonadotrophic complex.

[†] We have injected as high as 10 mg daily of purified lactogenic hormone into both normal mature and hypophysectomized female rats without stimulating the proliferation of mammary lobules.

12073

Sulfadiazine: Effect on *E. coli* Infections in Mice.

HARRY F. KLINEFELTER. (Introduced by P. H. Long.)

From the Department of Medicine and the Department of Preventive Medicine, The Johns Hopkins University, Baltimore, Maryland.

In a recent report,[1] it was shown that sulfathiazole is more effective than sulfanilamide or sulfapyridine in the treatment of *E. coli communis* infections in mice. Recently, the chemotherapeutic effect of sulfadiazine (2-sulfanilylpyrimidine) has been tested in experimental *E. coli communis* infections in mice, and the results are the subject of this report. The effects of sulfathiazole and sulfadiazine are compared.

Methods. The same strain of *E. coli communis* was used in this experiment as in the previous investigation.[1] The same media and methods of identifying the organism were employed. The pathogenicity of the bacterium for white mice (strain C F 1) remained the same; mucin enhanced its virulence so that less than 5 organisms injected intraperitoneally produced a fatal bacteremia within 24 hours.

Mice were infected intraperitoneally with 0.5 cc of a 1:500,000 dilution in mucin of a 12-hour broth culture of the test organism. The inocula, determined in the same way as in the previous report,[1] varied from 20 to 150 organisms, *i. e.,* from 20 to 100 M.L.D.'s.

The drugs were administered as part of the diet, constituting 1.0% of the diet with sulfathiazole and 0.1% with sulfadiazine. The animals were kept in separate cages and the amount of drug consumed each day was determined.

Results. A. *In vitro.* The bacteriostatic effect of sulfadiazine is compared with that of sulfathiazole in Table I. The method used

TABLE I.
Bacteriostatic Effect of Sulfadiazine and Sulfathiazole on *E. coli communis.*

Inoculum organisms per cc	Growth—Colonies per cc after 18-24 hr		
	Sulfadiazine 10 mg%	Sulfathiazole 10 mg%	Control
15	9,800,000	14,000,000	560,000,000
15	11,700,000	13,100,000	520,000,000
	50 mg%	50 mg%	
15	6,700,000	950,000	560,000,000
15	2,700,000	<100,000	520,000,000

---

[1] Klinefelter, H. F., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 365.

was similar to that described by McKee, *et al.*,[2] and Long and Bliss.[3]

In 10 mg % concentration, sulfadiazine is slightly more effective than sulfathiazole. In the higher concentration, both drugs exert more bacteriostasis than in the lower, but sulfathiazole is more effective than sulfadiazine.

B. *In vivo.* Mice were given diets containing 1.0% sulfathiazole or 0.1% sulfadiazine for 2 days before infection. On the morning of the third day, the mice that had eaten well of their diets were infected by the intraperitoneal route. The animals were then kept on their diets for 3 days following infection and observed for 7 more days before being discarded. The results are shown in Table II.

It is clear that sulfadiazine is more effective than sulfathiazole in treating *E. coli communis* infections in mice.

Feinstone, *et al.*,[4] have shown that sulfadiazine is more readily absorbed from the gastrointestinal tract of mice than is sulfathiazole. The blood levels in Table II were obtained from mice that had been kept on the drug diets for 5 or 6 days and confirm this observation. With an average daily intake of only one-tenth as much drug, the blood levels of the sulfadiazine mice are slightly higher than the levels of the sulfathiazole group.

Summary. 1. *In vitro* tests show that sulfadiazine has slightly more bacteriostatic action on *E. coli communis* than sulfathiazole in 10 mg % concentrations. 2. *In vivo* experiments show that, with comparable blood levels, sulfadiazine is more effective than sulfathiazole in treating *E. coli communis* infections in mice.

Conclusion. Sulfadiazine is the most effective of the sulfonamide

TABLE II.
Comparative Effect of Sulfadiazine and Sulfathiazole on *E. coli communis* Infections in Mice.

No. of mice	Inoculum colonies	Drug—% in diet	Avg daily intake, mg	Blood level, mg%		Deaths*—Hrs after infection			Survivals	
				Free	Total	24	48	72	No.	%
34	20-150	Sulfadiazine 0.1%	2.8	6.1	8.0				34	100.0
34	20-150	Sulfathiazole 1.0%	23.8	5.4	6.5	9	2	1	22	64.6
19	20-150	Controls				19			0	0.0

**E. coli communis* cultured from heart's blood in 100%.

---

[2] McKee, C. M., Rake, G., Greep, R. O., and Van Dyke, H. B., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 417.

[3] Long, P. H., and Bliss, E. A., *Ibid.*, 1940, **43**, 324.

[4] Feinstone, W. H., Williams, R. D., Wolfe, R. T., Huntingdon, E., and Crossley, M. L., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 427.

compounds in treating experimental colon bacillus infections in mice, and would seem to be the drug of choice in treating *E. coli* tissue infections in human beings.

12074 P

Studies on Soft Curd Milk Prepared by the Enzyme Treatment Method.

A. W. TURNER. (Introduced by Wm. H. Welker.)

From the Department of Physiological Chemistry, University of Illinois, College of Medicine, and Armour and Company, Chemical Research Laboratory.

This is a preliminary report on the investigation of the characteristics of enzyme-treated milk.[1]

The enzyme treatment[1] is carried out by adding one part of pancreatic concentrate, which has a high tryptic value, to 10 to 15 thousand parts of cold raw whole cow milk and then pasteurizing the enzyme-milk mixture immediately in the usual manner. The milk proteins are altered by the pancreatic enzymes as the temperature of the milk rises during pasteurization.

Methods. Amino nitrogen was determined by the Van Slyke gasometric method. All other nitrogen determinations were made with the Kjeldahl method. The precipitin reaction was carried out according to the technic of Hektoen and Welker.[2] All control milk samples were prepared identically the same as the enzyme-treated milk samples and from the same milk without enzyme being added. The technic of the digestibility test which was found to be satisfactory is as follows:

Eight hundred ml of milk at 37°C is mixed with 80 ml of .5 N HCl containing .056% U.S.P. pepsin. The resulting pH is 4.8. Fifty ml portions are removed and filtered after 5 and 30 minutes' digestion at 37°C with mild agitation. The filtrates are set aside for total N analysis. The digest is then neutralized to pH 7.5 with NaOH and .112 g of U.S.P. trypsin is added. After 15 minutes of mild agitation at 37°C a third 50 ml portion is removed and adjusted to isoelectric pH 4.8 with HCl and then filtered to get the noncoagulable isoelectric protein filtrate. This is repeated every 20 minutes thereafter until a total digestion time of about 2½ hours

---

[1] Conquest, V., Turner, A. W., and Reynolds, H. J., *J. Dairy Sci.*, 1938, **21**, 361.

[2] Hektoen, Ludwig, and Welker, Wm. H., *J. Infect. Dis.*, 1924, **35**, 294.

TABLE I.
Comparison of the *In Vitro* Digestibility of Enzyme-Treated and Pasteurized Whole Cow Milk.

Time of digestion min.	g protein per 100 cc filtrate	
	Pasteurized whole milk	Enzyme-treated whole milk
5	.84	.96
30	.96	1.13
45	1.13	1.48
65	1.36	1.62
85	1.61	1.92
115	1.75	2.01
150	2.05	2.27

has elapsed. During the digestion, a pH level of 7.5 is maintained by the addition of NaOH. This test was used to study the digestibility of enzyme-treated milk.

Results. It was found that the enzyme-milk filtrates contained an average of 18.1% more protein than the control milk filtrates in 6 trials.

Table I shows the results of a typical digestibility test.

Enzyme-treated milk samples and their controls were made up to a 70% alcohol content, permitted to stand at room temperature over night and filtered. Total nitrogen was then determined on the filtrates to obtain the amount of alcohol-soluble protein in the milk. Table II shows that the enzyme treatment increases the amount of alcohol-soluble protein in whole milk.

No significant increase in the amino nitrogen content of enzyme-treated milk was observed. See Table II for the analytical results.

Proteose and peptone nitrogen of enzyme-treated milk and its control was measured by running total nitrogen on the trichloracetic acid filtrates of the milk. See Table II for the analytical data of this experiment.

These results do not show a significant increase in proteose nitrogen of enzyme-treated milk, but they do indicate a definite higher value.

Casein was prepared and purified from enzyme-treated skim milk

TABLE II.

Nitrogen fraction determined	g N per 100 ml milk		No. of trials
	Enzyme-treated whole milk	Regular pasteurized whole milk control	
Alcohol soluble protein	.174	.109	7
Proteose peptone	.0896	.0572	3
Amino acid	.019	.016	3

TABLE III.
The 70% Alcohol Solubility of Enzyme-Treated and Regular Pasteurized Skim Milk HCl and H_2SO_4 Caseins.

Type casein	g protein per 100 ml alcoholic filtrate Enzyme-treated casein	 Pasteurized casein control	No. of trials
HCl casein	.78	.44	5
H_2SO_4 casein	.079	.044	4

samples and their controls by precipitation with HCl or H_2SO_4 at 45°C. The curd was then washed 5 times with water, redissolved at pH 8.0 with NaOH, reprecipitated with acid, washed, dried at 45°C, and ground to 20 mesh size. Forty grams of the casein were then suspended in 250 ml of 70% alcohol for 3 days at room temperature. The undissolved casein particles were filtered from the alcohol and total nitrogen was determined on the alcoholic filtrate to estimate the solubility of the caseins in 70% alcohol. Table III shows the analytical data obtained in this experiment.

These results demonstrate the increased solubilities of enzyme-treated HCl and H_2SO_4 caseins in 70% alcohol.

Immunologic studies of 70% alcohol-soluble protein from raw skim milk showed that the immune sera obtained from rabbits immunized with this protein gave positive precipitin tests with enzyme-treated whole and skim milk, raw whole milk, raw whole milk rennet whey, pasteurized whole milk rennet whey, 70% alcohol-soluble protein from enzyme-treated skim milk, 70% alcohol-soluble protein from pasteurized skim milk, sweet cream dried buttermilk, and spray dried skim milk. Similarly these same immune sera gave negative precipitin tests with purified ox albumin, ox pseudoglobulin, lactoglobulin, lactalbumin and purified 70% alcohol-soluble protein prepared from pasteurized skim milk HCl casein.

Seventy percent alcohol-soluble protein from pasteurized skim milk HCl casein was used in an attempt to immunize rabbits. Excessive and repeated doses in 2 rabbits failed to produce sera giving positive results in the precipitin test.

The observed differences between pasteurized and enzyme-treated cow milk are as follows: Enzyme-treated milk contains more 70% alcohol-soluble protein and proteose peptone nitrogen, but only slightly more amino nitrogen than ordinary pasteurized milk. Casein prepared from enzyme-treated skim milk is more soluble in 70% alcohol than casein prepared from pasteurized skim milk.

12075 P

Relation of Respiratory Ventilation to Basal Metabolism in Thyroid Disorders.

F. W. WEYMOUTH AND ARTHUR T. BRICE.

From the Department of Physiology, Stanford University.

An analysis of basal metabolism records made available to one of us (ATB) from the files of the United States Veterans' Facility, Fort Miley, California, shows a relation between respiratory ventilation and metabolism which we believe has not been reported previously. The coefficient of ventilation (CV), or volume percent removed as oxygen from the tidal volume,[1] has been used as a measure of respiratory activity. All data were obtained by one of us (ATB), using a conventional closed-circuit metabolism apparatus, with 16-minute recording periods.

Ventilation, due to the mechanism of control normally depending on the CO_2 content of the blood, makes available, under ordinary circumstances, a considerable surplus of oxygen over the metabolic needs. Thus at rest the average man uses only 4% of the tidal volume ($CV = 4$) or about one-fifth of the oxygen available. This factor of safety of 5 for oxygen may be increased to 20 by over-ventilation, that is, the CV may fall as low as one. The data contained 76 records from 37 persons diagnosed as suffering from various thyroid disorders. Combining all types the BMR ranged from –54% to 114% (Aub-DuBois standards). The CV for a group of 14 having "normal" BMR (mean +0.857%) is 4.393, slightly above the 3.996 found for normal men. With either raised or lowered metabolism the value of the CV falls in a degree roughly proportionate to the severity of the metabolic disturbance. The means of the groups are given in Table I. The falling trend in either direction is clear but the variability is so great that the reliabilities must be considered. The probabilities (Fisher's t method), expressed as chances per hundred, that a difference as great or greater than that recorded would arise by random sampling from a population in which no difference existed are given in Table II. The average CV of persons with normal BMR is doubtfully above the mean of the group with lowered metabolism ($P = 10$ times in 100). The normals are not significantly above the group having the next higher BMR but are significantly higher than the second group (1.5 in 100). It is thus clear that the fall of CV with raised metabolism

---

[1] Zeigler, E. E., *Med. Annal. Dist. Columbia*, 1933, **2**, 225.

TABLE I.
Mean Coefficients of Ventilation for Various Levels of Basal Metabolic Rate.

No.	BMR%	CV
12	— 24.500	+3.550
14	+ 0.857	+4.393
18	+ 18.833	+3.511
15	+ 35.467	+3.100
10	+ 59.200	+2.340
2	+ 79.000	+2.200
2	+107.500	+2.100

TABLE II.
Probabilities for Various Intercomparisons of Coefficients of Ventilation.

Values of CV compared		Probability, chances per hundred
4.393	3.550	10.0
4.393	3.511	20.0
4.393	3.100	1.5
3.511	3.100	50.0
3.100	2.340	15.0

is statistically significant, and that with lowered BMR, suggestive. More data might be expected to make this significant. One retest on an individual tends to support this conclusion. A man suffering from hypothyroidism and using thyroid extract for many years, entered the hospital for a periodic checkup. The oxygen consumption was 285 ml (standard conditions) per minute (BMR –1%) and the respiratory minute volume 6400 ml; the CV, 4.5. When thyroid administration was stopped for 10 days the BMR fell to –26% (212 ml per minute) while the respiratory minute volume rose to 12,900 ml, slightly more than twice the original volume, giving a CV of 1.6. Both times the test was in duplicate. This is a clear case of great overventilation accompanying a marked fall of metabolism known to be due to reduced thyroid influence.

To summarize, deviations in either direction from normal metabolism due to thyroid dysfunction are accompanied by overventilation. This fall is statistically valid for increased and highly probable for decreased BMR. The variability is so great, however, that this trend may fail to show in many individual observations. If we assume with Simonson[2] that the degree of overventilation is a measure of the sensitivity of the respiratory center, we would conclude that both excess and deficiency of the thyroid secretion increases the sensitivity of this part of the central nervous system. This is generally accepted as true in man of the hyperthyroid but not of the hypothyroid condition.

---

[2] Simonson, E., *Pflügers Arch.*, 1926, **214**, 380; 1926-27, **215**, 752.

12076

Cause of Death of Toads after Destruction of their Lymph Hearts.

Virgilio G. Foglia. (Introduced by Chauncey D. Leake.)
From the Institute of Physiology, Faculty of Medical Sciences of Buenos Aires.

Destruction of the 4 lymph hearts of batrachians produces marked disturbances in lymph circulation, leading to rapid death[1, 2] as we have repeatedly demonstrated in some 500 toads (*Bufo arenarum* Hensel) and frogs (*Leptodactyllus ocellatus* (L.) Gir.). The anterior and posterior lymph hearts are easily destroyed by dorsal route approach under ether anesthesia and cauterizing with a thermocautery. The operation requires 2 to 3 minutes and when the effects of anesthesia have worn off, the animals appear entirely normal, and may be put in sinks kept damp by running water.

All the toads in which the 4 lymph hearts were destroyed at one operation died before the 4th postoperative day in winter, and before the 3rd in summer. Mortality during the first 2 days was 87% of all operated animals. Longer survival seems due to incomplete operation.

The weight of the toads was found to increase progressively from operation to death at the rate of 20% daily. At necropsy, an enormous accumulation of fluid was observed in the lymph spaces, the peritoneal cavity, and the tissues. This fluid retention seems to be enough to account for the increase in weight of toads (Table I).

Presence of a single lymph heart seems sufficient to maintain toads for an indefinite period of time without apparent symptoms. It was observed that if 3 of the lymph hearts were destroyed at one

TABLE I.
Change in Body Weight and Mortality of Toads without Lymph Hearts and of Normal Controls Kept in Sinks Moistened with Running Water.

	Avg time of death of							
	38 toads without lymph hearts				28 normal control toads			
At end of day	1	2	3	4	1	2	3	4
Daily mortality %	21	66	10	3	0	0	0	0
Increase in wt (% of initial wt)	20	38	60	—	0	0	0	0

---

[1] Foglia, V. G., *Rev. Soc. Argent. Biol.*, 1939, **15**, 97; *Compt. rend. soc. biol.*, 1940, **133**, 153.

[2] Foglia, V. G., and Gerschman, R., *Rev. Soc. Argent. Biol.*, 1939, **15**, 113; *Compt. rend. soc. biol.*, 1940, **133**, 155.

operation and the fourth a week later, survival was more prolonged than when all 4 hearts were destroyed simultaneously. In one group of 7 toads, operated upon in 2 stages, 2 animals continued to live for 2 months while the others survived for 2, 5, 9, 11, and 12 days respectively. This prolonged survival was accompanied by little or no increase in body weight. It is possible that in these cases the ciliated funnels of the peritoneum, well studied by Rugh,[3] permitted the establishment of an active circulation between lymphatics and veins.

The studies of Pascualini[4] on absorption of substances injected under the skin, have demonstrated that the reabsorption of fluid takes place by way of the lymph hearts and blood capillaries, the former being more important. Substances of high molecular weight such as inulin and hemoglobin pass into the circulation by way of the lymph hearts. On the other hand, those of low molecular weight such as glucose, sodium chloride, and strychnine pass both by way of the blood capillaries and the lymph hearts. Destruction of the lymph hearts therefore impedes the absorption of substances of the first group only.

After paralysis of the posterior lymph hearts by cutting their nerves, accompanied by cauterization of the anterior lymph hearts, toads die in the same period of time and with the same signs as when there is destruction of all 4 hearts. Mechanical obstruction of the lymphatic circulation, due to operation does not, therefore, produce the disturbances, but the cessation of contraction of the hearts is the cause of death.

It was not found possible to prolong life of toads with all the lymph hearts destroyed by keeping them in a dry or a humid atmosphere, or by submerging them in distilled or tap water or in solutions of sodium chloride in concentrations varying between 8 and 10%.

In toads with all lymph hearts destroyed, profound alterations in blood, lymph, and tissues occur. These begin at the time of operation and increase progressively until death. Our observations on these changes are summarized in Table II.

Observed changes in the blood seem to depend directly upon the enormous loss of water and salts (chlorides, sodium, and potassium). Their loss causes (a) a diminution in blood volume; (b) the appearance of hemolysis which increases progressively post-operatively; (c) marked hemoconcentration as evidenced by an increase in the hematocrit, red cells per cubic millimeter, hemoglobin, and total

---

[3] Rugh, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 717.

[4] Pascualini, R., *Rev. Soc. Argent. Biol.*, 1939, **15**, 438.

TABLE II.
Chemical Changes Observed in Toads (*Bufo arenarum* Hensel) Kept in Moist Sinks Before and After Destruction of the 4 Lymph Hearts.
Average Values of Several Groups of 40 Toads Each.

	g%		mg%					
	Water	Total nitrogen	Chlorides	Sodium	Potassium	Hematocrit, %	Hemoglobin, g%	Erythrocytes per mm3
Blood before	84.4	1.52	263	233	145	33.4	8.6	
24 hr after	76.8	3.33	202	152	175	51	16.8	
36 ” ”			121	100	135	68.6		
Plasma before		0.55	335	305	13			
24 hr after		0.59	282	250	21			
36 ” ”			198	198				
Erythrocytes before		1.53	157	120	370			710,000
24 hr after		3.33	128	73	254			810,000
36 ” ”			92	80	150			1,250,000
Lymph, 36 hr after	97.5	0.3	187	167	21			
Liver before	68.8	2.3	131		420			
24 hr after	70.2	2.1	67		229			
Muscle before	77.1	3	60		305			
24 hr after	81.5	2.5	49		290			

nitrogen (which is due especially to the increase in concentration of hemoglobin). As a consequence of these alterations in the blood, it is observed that (a) the mean arterial aortic pressure decreases about 30%; (b) the glomerular and capillary circulation of the kidney is sluggish; and (c) urinary output is only a third of that of the controls.

In the tissues (liver and muscles), there is an important loss of chloride and potassium. The increase in water content and decrease in protein content observed in the direct analysis of muscle must be interpreted as resulting from interstitial edema. The enormous amount of lymph which accumulates consists of 97% water. There is a protein content of 2.05%. The salts (chlorides, sodium, and potassium) are in a concentration equal to or slightly less than that of blood. These mineral elements come from blood and tissues.

In summary, acute destruction of the lymph hearts of the toad (*Bufo arenarum* Hensel) and the frog (*Leptodactyllus ocellatus* L. Gir.) leads to death within 4 days. During this time, body weight increases and edema fluid appears in the lymphatic spaces. In their development, the disturbances of the venous lymphatic circulation produced by the abrupt cessation of contraction of the lymph hearts play a primary rôle, for paralysis of the lymph hearts by denervation causes death. These disturbances can be overcome and death avoided by destroying the hearts in 2 stages separated by an interval of at least a week which gives time for the development of new collateral pathways. The existence of a single intact lymph heart prevents the development of all disturbances, including death. As a result of the destruction of the lymph hearts, the lymph which transudes from the blood vessels collects in the lymph spaces and cannot return to the blood stream. Water, salts, and proteins coming from blood and tissues remain in the lymph. Water in large amounts comes from the external environment. The decrease in the content of water, salts, and possibly protein in the blood and the changes in the chemical composition of the tissues cause the death of the animal.

I wish to express my thanks and appreciation to Dr. B. A. Houssay, whose aid and advice have made this work possible.

12077 P

Transmission of St. Louis Encephalitis to the Hamster.

G. O. BROUN, R. O. MUETHER, R. A. MEZERA AND M. LEGIER.
From the Department of Medicine, St. Louis University School of Medicine.

The virus of St. Louis encephalitis has been found to be pathogenic for relatively few laboratory animals. Muckenfuss, Armstrong and McCordock[1] first established the virus in monkeys, but it was difficult to maintain in this animal. Webster and Fite[2] showed that Swiss mice could be infected easily by intracerebral and intranasal inoculation. All types of albino mice as well as house mice,[3] field mice and meadow mice[4] are susceptible.

Smith[5] has shown that the virus of St. Louis encephalitis persists in the brain of rats and guinea pigs for 8 or 9 days after intracerebral inoculation and slight anatomical lesions are demonstrable. These animals, however, showed no symptoms of disease. The virus is demonstrable in rat and guinea pig brain only after the first transfer. She concludes that the virus has not been established in mice and guinea pigs by serial passage. Sulkin, Harford and Bronfenbrenner[6] carried the virus of St. Louis encephalitis through 6 successive passages in guinea pigs and the following year through 5 successful passages. However, later they were unsuccessful in carrying the virus in this animal. Our own experience with inoculations of rats and guinea pigs is similar to that of Smith.[5] We have had on a few occasions death of inoculated rats on the sixth or seventh day after inoculation and were able to recover the virus from the brains of these animals. However, most animals proved resistant and serial transfer of virus from animal to animal was not successful.

---

[1] Muckenfuss, R. S., Armstrong, C., and McCordock, H. A., *Pub. Health Rep.*, 1933, **48**, 1341.

[2] Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

[3] Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 331.

[4] Greutter, J. E., Fulton, J. D., Muether, R. O., Hanss, E. V., and Broun, G. O., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 253.

[5] Smith, M. G., *J. Infect. Dis.*, 1939, **64**, 307.

Since studies of the serological aspects of St. Louis encephalitis would be facilitated by the discovery of a susceptible animal of somewhat larger size than the mouse, we have recently tried the effect of inoculation of the virus into the golden or Syrian hamster, *Mesocricetus auratus.*

This animal is only slightly smaller than the guinea pig. It can be readily propagated for laboratory use. Blood can be withdrawn from the heart in sufficient quantities to permit serological studies of individual animals.

We immediately found that this animal is highly susceptible to infection with the virus of St. Louis encephalitis both by intracerebral and intranasal inoculation. The animals show evidences of illness, such as weakness, somnolence and tremors on the third and fourth days after intracerebral inoculation. Usually within 24 hours after appearance of such symptoms the animal dies. Microscopic evidence of encephalitis is found on pathological examination of the brain. The presence of the virus can be shown by virus neutralization tests. Its concentration is very close to that found in the brains of mice.

After intranasal inoculation illness occurs on the fifth or sixth day with death taking place approximately one day later. Subcutaneous inoculation is not followed by evidence of illness. The results of oral administration are under study.

The virus has been carried through 4 serial transfers from animal to animal without loss of virulence.

We already have evidence that neutralizing antibodies may be demonstrated in the blood of these animals. Their time of appearance under various circumstances is now under investigation.

---

[6] Sulkin, S. E., Harford, C. G., and Bronfenbrenner, J., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 329.

Summary. The Syrian hamster is highly susceptible to intracerebral and intranasal inoculation with the virus of St. Louis encephalitis.

12078

Anemia in Vitamin C Deficiency and Its Response to Iron.

S. H. Liu, H. I. Chu, T. F. Yu, H. C. Hsu and T. Y. Cheng.

From the Department of Medicine, Peiping Union Medical College, Peiping.

Experimental scurvy in the guinea pig is usually associated with anemia,[1] and antiscorbutic food[2] or vitamin C[3] improves the anemia together with the scurvy. In human nutrition, hypochromic anemia frequently accompanies scurvy in infants,[4] and it is also commonly found in adults with vitamin C deficiency. Mettier, Minot and Townsend[5] postulate that vitamin C exerts a specific effect on erythropoiesis. Dunlop and Scarborough[6] reported 2 cases of scurvy with anemia which disappeared on supplementing a deficient diet with 60 mg of ascorbic acid daily. On the other hand, Keefer and Yang[7] state that in man scurvy may exist without anemia, although, if the disease is of considerable duration and associated with hemorrhage, infection or general undernutrition, anemia may be present. Likewise, Abt and Farmer[8] feel that when anemia accompanies lack of vitamin C, it is probably due to a generally deficient diet in which substances other than vitamin C, especially iron, are lacking. The recent experimental production of scurvy in a human subject without appearance of anemia[9] also supports the latter viewpoint.

In view of the existing controversy in regard to the rôle of vitamin C in anemia, the following data obtained in the Spring of

---

[1] Meyer, A. W., and McCormick, L. W., *Stanford Univ. Pub. Med. Sc.*, 1928, **2**, 96, 199.

[2] Mettier, S. R., and Chew, W. B., *J. Exp. Med.*, 1932, **55**, 971.

[3] Aron, H. C. S., *J. Nutrition*, 1939, **18**, 375.

[4] Hess, A. F., *Scurvy, Past and Present*, Philadelphia, Lippincott Co., 1920; Parsons, L. G., and Hawksley, J. C., *Arch. Dis. Child.*, 1933, **8**, 117.

[5] Mettier, S. R., Minot, G. R., and Townsend, W. C., *J. Am. Med. Assn.*, 1930, **95**, 1089.

[6] Dunlop, D. M., and Scarborough, H., *Edinburgh Med. J.*, 1935, **42**, 476.

[7] Keefer, C. S., and Yang, C. S., *Nat. Med. J. China*, 1929, **15**, 419.

[8] Abt, A. F., and Farmer, C. J., *J. Am. Med. Assn.*, 1938, **111**, 1555.

[9] Crandon, J. H., Lund, C. C., and Dill, D. B., *New England J. Med.*, 1940, **223**, 354.

1939 in the course of a nutritional survey of 238 boys in a municipal relief institution may be of interest. These boys, ranging from 11 to 20 years of age, lived on a monotonous diet of millet and corn with small amounts of vegetables in season. Most of them appeared to be underdeveloped and sparely nourished. Sixty of them had pallor, 112 had hypertrophied and bleeding gums and 87 had positive Chvostek's sign. However, none showed bleeding tendency elsewhere, manifest tetany, ocular or cutaneous manifestations of avitaminosis A or neurological evidence of vitamin B_1 deficiency.

Of this series, 44 inmates who had hypertrophied gums were selected for more detailed study. Their Pelidisi indices varied between 87 and 99, averaging 92. Twenty-six had underdeveloped external genitalia. Eighteen showed latent tetany, as evidenced by Chvostek's or Trousseau's sign or both. Three were suspected to have pulmonary tuberculosis, 3 had enlarged liver and 4 enlarged spleen. The plasma vitamin C values were all low : 28 cases ranging between 0.10 and 0.19, 9 cases between 0.20 and 0.29, and 7 cases between 0.30 and 0.40 mg per 100 cc. However, only 21 cases showed increased capillary fragility by the Gothlin's test. Anemia was present in more than half of the cases, the hematocrit reading being from 27.2 to 30% in 3 cases, from 30 to 40% in 30 cases and from 40 to 45.6% in 16 cases. The anemia observed was mostly of the hypochromic type, with considerable achromia and anisocytosis. No nucleated erythrocytes were noted in the peripheral blood. Platelet counts varied between 210,000 and 333,000 per cu mm, and reticulocyte counts between 0.7 and 3.2%. Bleeding time, coagulation time and prothrombin time (Quick) were all normal.

In order to determine the cause of the anemia, we selected for therapy 16 boys of the above series who had varying degrees of anemia without apparent explanation. They were evenly divided into 2 groups. The subjects in the first group each received 50 mg of vitamin C (Cebion, Merck) daily for 4 weeks, and those of the second group 1.5 g of ferrous carbonate daily for the same length of time. During the period of treatment, the boys remained in the relief institution without any change in their diet or routine. Hematological studies and plasma vitamin C determinations made before and after the medication are set forth in Table I. The observations before and after therapy have been paired and tested for significance by means of the *t*-test,[10] and probability values of 0.05 or less are considered significant.

---

[10] Fisher, R. A., *Statistical Methods for Research Workers*, 7th ed., 1938, Oliver and Boyd, London.

TABLE I.

Case No.		Hematocrit % cell volume	Erythrocyte million per mm^3	Hemoglobin g per 100 cc	Mean corpuscular vol., μ^3	Mean corpuscular hgb. 10^{-12} g	Mean corpuscular hgb. conc., %	Reticulocyte, %	Plasma vitamin C mg%
				Vitamin C-Treated Series.					
1	Before	20.0	2.23	5.1	89.7	22.8	25.5	1.8	0.38
	After	18.3	2.23	5.2	82.0	23.3	28.4	2.0	1.40
2	Before	28.3	3.40	7.9	83.3	23.2	27.9	1.8	0.12
	After	28.1	3.43	7.9	82.0	23.0	28.1	1.7	0.98
3	Before	34.2	2.93	9.9	116.6	33.7	28.9	2.8	0.29
	After	34.3	3.22	10.4	106.5	32.3	30.4	2.4	0.97
4	Before	35.4	3.81	10.6	92.9	27.8	30.0	1.4	0.15
	After	32.9	3.08	10.0	106.7	32.5	30.4	3.1	0.73
5	Before	35.3	3.76	10.9	94.8	28.9	30.9	1.0	0.13
	After	35.5	2.98	11.8	119.0	39.6	33.3	2.5	0.49
6	Before	35.9	3.22	11.0	111.5	34.2	30.7	3.2	0.25
	After	35.0	3.36	10.1	104.0	30.0	28.9	2.3	1.27
7	Before	36.1	3.89	11.8	92.8	30.3	32.7	1.0	0.35
	After	34.8	3.16	11.6	110.0	36.7	33.3	1.8	0.92
8	Before	38.0	3.14	12.1	121.0	38.5	31.8	1.8	0.15
	After	38.0	2.70	12.9	140.0	47.5	33.9	2.1	0.50
Mean	Before	32.9	3.30	9.9	100.3	30.0	29.8	1.8	0.23
	After	32.1	3.02	10.0	106.2	33.1	30.8	2.0	0.91
	t	0.22	1.68	0.44	1.60	1.70	1.93	1.07	7.09
Probability		0.8	0.1	0.7	0.2	0.1	0.1	0.3	0.001
				Iron-Treated Series					
1	Before	27.2	3.71	6.9	73.2	18.6	25.4	2.6	0.38
	After	38.0	4.22	13.1	90.0	31.0	34.5	2.8	0.22
2	Before	27.8	2.96	6.8	94.0	22.9	24.4	1.2	0.14
	After	33.2	3.68	10.1	90.4	27.4	30.4	1.7	0.23
3	Before	34.1	2.56	9.1	133.1	35.5	26.7	2.0	0.18
	After	40.2	3.99	12.2	100.7	30.6	30.3	2.1	0.27
4	Before	33.8	2.99	9.7	113.0	32.4	28.7	2.0	0.12
	After	37.0	3.93	12.6	94.0	32.1	34.1	1.9	0.22
5	Before	31.8	3.27	9.0	97.3	27.5	28.3	1.0	0.12
	After	36.2	3.25	11.8	111.2	36.3	32.6	2.7	0.22
6	Before	38.3	3.19	11.8	120.0	37.0	30.7	1.8	0.21
	After	42.3	4.06	14.5	104.0	35.7	34.3	2.1	0.21
7	Before	35.2	3.84	9.7	91.7	25.2	27.5	1.4	0.18
	After	36.5	4.20	12.3	86.8	29.3	33.7	2.4	0.17
8	Before	34.2	3.11	11.0	110.0	35.4	32.2	2.5	0.17
	After	34.7	2.62	11.2	132.5	42.7	32.2	—	0.19
Mean	Before	32.8	3.20	9.2	103.0	29.3	28.0	1.8	0.19
	After	37.3	3.74	12.1	101.2	33.1	32.8	2.2	0.22
	t	3.77	2.51	5.26	0.38	1.87	5.16	0.21	0.94
Probability		0.01	0.05	0.001	0.7	0.1	0.001	0.8	0.4

It will be noted that vitamin C administration, while capable of raising the plasma vitamin C concentration in every instance with an average increase from 0.23 to 0.91 mg %, failed to influence the hematocrit or hemoglobin values. The slight reduction in the average erythrocyte count after vitamin C therapy was not significant, likewise the changes in the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were insignificant. There was no significant rise in reticulocyte percentage following vitamin C medication. On the other hand, iron therapy was followed in most cases by a significant increase in hematocrit, erythrocyte and hemoglobin values, although, as expected, the plasma vitamin C concentration remained subnormal as before in all instances. The average increase of hematocrit was from 32.8 to 37.3%, that of erythrocyte count from 3.20 to 3.74 million per cu mm, and that of hemoglobin from 9.2 to 12.1 g per 100 cc. The mean corpuscular volume and the mean corpuscular hemoglobin showed insignificant variations, while the mean corpuscular hemoglobin concentration evinced a definite increase after the iron administration. No distinct rise in reticulocyte count was noted.

Summary. Anemia appears to be common in association with vitamin C deficiency among the inmates of a municipal relief institution studied. Therapeutic results indicate that the anemia is not due to lack of vitamin C *per se,* but related in all probability to a concomitant iron deficiency.

12079

Attempts to Grow *Leishmania donovani* in Tissue Cultures.

H. C. PAI AND C. H. HU.

From the Department of Pathology, Peiping Union Medical College, Peiping, China.

The parasites of kala-azar, *Leishmania donovani,* live as non-motile forms only in the cells of the mammalian hosts. When they are cultured in the ordinary artificial media at 37°C, no growth occurs, but at room temperature, 22-25°C, or when brought to the digestive tract of certain insects, they develop into motile flagellates. It seems, therefore, that in order to cultivate the oval non-motile form of the parasites *in vitro,* an opportunity must be given them to grow in the living cells, and the tissue-culture method naturally suggests itself.

Gavrilov and Laurencin[1] have already made use of this method to cultivate these parasites, using the tissue of the infected hamsters. They found that the oval forms survived in the cells for only about 10 days. The following experiments were undertaken to determine whether it is possible to get a more successful growth of these parasites in the tissue cultures.

---

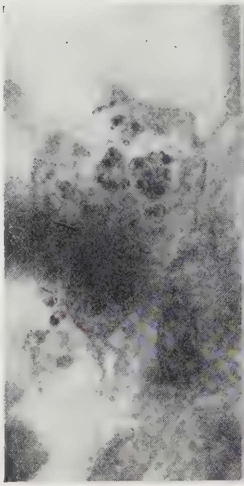
[1] Gavrilov, W., et Laurencin, S., *Ann. Soc. Belge Med. Trop.*, 1938, **18**, 1.

Two series of experiments were made with the tissue cultures kept at 37°C. In the first series, 51 explantations by the hanging drop method were made of the spleen and the calf muscle of chicken embryos, the spleen, the omentum and the subcutaneous tissue of normal adult hamsters and hamsters infected with kala-azar, and the spleen and the subcutaneous tissue of a human fetus. For the chicken embryonic tissues, chicken plasma and embryonic tissue juice were used as media. For the hamster and human tissues, hamster serum and human serum from the umbilical cord respectively were added to the above mentioned media. After 3 to 7 transplantations, a drop of the suspension of *Leishmania donovani* (obtained from the spleen of an infected hamster) in Tyrode's solution was introduced into, and thoroughly mixed with, the media except in cases in which the tissue used was already infected. Transfers were subsequently made every 2 to 3 days, whole cultures were fixed and stained with iron hematoxylin at different intervals. It was found that within 24 hours the parasites were already taken up by large roundish phagocytes which contained from one to 15 parasites in their cytoplasm (Fig. 1). In some cultures the parasites began to degenerate on the seventh or eighth day of cultivation; in others they remained normal in appearance even on the thirteenth day, but by the fifteenth day all the parasites were found to be degenerated. It was also found that frequent transfer hastened the degeneration of the parasites and that different kinds of tissues yielded practically the same results.

In the second series of experiments only the calf muscle of the chicken embryo was employed. Altogether 65 explantations were made. In order to keep the tissue culture growing at a slower rate, the tissue, after being infected with *Leishmania donovani* in the hanging drop culture, was transferred into a Carrel flask prepared according to the method of Fischer and Parker[2] in which was used a solid medium composed of about 0.5 cc of chicken plasma and 1 cc of Tyrode's solution with only a very small amount of embryonic tissue juice to hasten the coagulation. The cultures were subsequently washed with Tyrode's solution and fed with heparinized plasma every 2 or 3 days. At different intervals the cultures were sectioned and stained for histological study. It was found that the parasites began to show degeneration after 20 days of cultivation; but some of them were morphologically and tinctorially still normal on the 35th day; by the 43rd day, however, only the degenerated forms were seen. Evidence of multiplication of the

---

[2] Fischer, A., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 585.



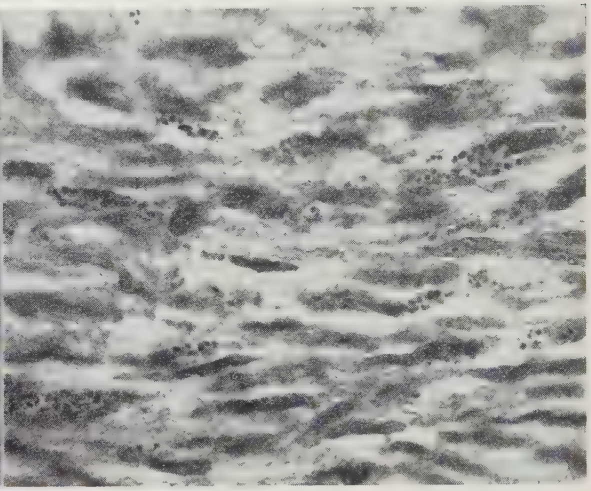


FIG. 1. FIG. 2.

FIG. 1. Photomicrograph showing the still well preserved *Leishmania donovani* in a phagocyte, 12 days after cultivation in a hanging drop culture. Iron hematoxylin stain. × 1000.

FIG. 2. Photomicrograph showing the normal appearing *Leishmania donovani* in elongated fibroblast-like cells, 35 days after cultivation in a Carrel flask. Tissue was sectioned and stained with hematoxylin and eosin. × 745.

parasites was at no time observed. In the older cultures the parasite-containing cells had transformed into elongated fibroblast-like cells (Fig. 2).

Summary and Conclusion. Attempts to grow *Leishmania donovani* were made in cultures of chicken, hamster and human tissues. It was found that the parasites were taken up by the phagocytes within 24 hours. In the hanging drop cultures parasites were all degenerated by the 15th day. In the slowly growing cultures in flasks prepared according to Fischer and Parker's method they were all degenerated by the 43rd day. Multiplication of the parasites has not been observed.

It is concluded that the parasites may survive for various lengths of time but cannot grow in the tissue cultures according to the methods described, and that the length of survival is in inverse ratio with the rapidity of the growth of the tissue cultures.

12080 P

Isolation of a Virus from an Acute Encephalitis Case in Peiping.

C. H. Yen. (Introduced by Samuel H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

While the recent occurrence of acute encephalitis in North China has attracted some attention of the clinicians,[1] the etiological agent has hitherto not been isolated. In view of the epidemiological interest it seems of importance to determine whether the etiology of acute encephalitis encountered locally is a known virus such as the Japanese type B and the St. Louis type, or a new type of virus. In the present communication observation made on a virus isolated from a case of acute encephalitis is briefly described.

The patient, a Chinese boy, 9 years of age with a 2-day history of fever, repeated vomitings and attacks of convulsions, was admitted to the Peiping Union Medical College Hospital on September 23, 1940. Blood and spinal fluid cultures made at this time failed to show growth of organisms. The spinal fluid was slightly cloudy and contained in one cu mm, 46 white blood cells which are chiefly lymphocytes. The patient died on the 4th day of fever. On postmortem examination essentially normal findings except for the gross appearance of congestion of the vessels of the central nervous system were seen. Sections of the brain showed presence of widely scattered areas of perivascular infiltration with lymphocytes. A portion of the brain was ground with sterile sand and made up to 10% suspension with sterile meat infusion broth (pH 7.6). The suspension was then centrifuged at 3,000 r.p.m. for 15 minutes. 0.025 cc of the supernatant was inoculated intracerebrally and separately to 7 Swiss mice, each weighing about 15-17 g. Beginning from the 5th day after inoculation, 2 of the animals began to show tremor, spasm and weakness of the limbs which became paralyzed on the 6th day and finally died on the 7th day; the remaining 5 became sick with roughened hair and hunched body without showing tremor or paralysis and died between 5th and 7th days. Paraffin sections of brains from animals dying from the infection showed numerous areas of perivascular lymphocytic infiltration. The choroid plexuses, meninges and the visceral organs appeared normal.

---

[1] Kuttner, A. G., and T'ung, T., *J. Clin. Invest.*, 1936, **15**, 525; Chu, F. T., Wu, J. P., and Teng, C. H., *Chinese Med. J.*, 1940, **58**, 68; Huang, C. H., and Liu, S. H., *Chinese Med. J.*, 1940, **58**, 427.

Brain from the infected mice dying from 5-7 days was found to be infective for normal Swiss mice, as little as 0.025 cc of a 1:100,000 dilution of a brain injected intracerebrally being sufficient to cause death of these animals in 5-7 days, usually accompanied with symptoms of tremor or paralysis. By intracerebral inoculations the infection has been passed from mouse to mouse for more than 12 generations. In all instances the infection regularly occurred and the same pathological findings as those found in the primary mouse passage were present. On several occasions the infected brain suspensions were first filtered through "V", "N" and "W" Berkefeld filters or Seitz E.K. pads before intracerebral inoculations were made. The filtrate produced the same kind of picture of infection in Swiss mice as those caused by the unfiltered suspensions. The infective agent has remained potent when kept in 50% glycerin for 2 months although a drop of the infective titer was seen after 3 weeks of preservation. In ordinary broth suspensions, however, the infectivity rapidly deteriorated in a few days. It seems clear that the infective agent recovered from the brain of the encephalitis patients behaved like one of the filterable viruses, and is therefore designated as Peiping Encephalitis Virus No. 1 (P. E. No. 1).

Rabbits, guinea pigs, white rats and Chinese hamsters were found to be resistant to the P. E. virus either by intracerebral or intra-abdominal inoculations with the infected mouse brain. However, no attempt was made to establish the infection by repeated animal to animal passage in these species.

A monkey *(Macacus rhesus)* was found to be susceptible to intracerebral inoculation with this virus; the animal developed fever and tremor after an incubation of 6 days and finally died on the 10th day.

By repeated intraäbdominal inoculations with the infected mouse brains, rabbits failed to succumb to the infection but developed potent antiviral antibodies in their sera. By similar procedures, neutralizing sera against Japanese type B. S.K. (Hashimoto)* and St. Louis No. 3 (Webster)* encephalitis viruses were separately prepared from rabbits for testing with the Peiping encephalitis virus. It was found that the Japanese S.K. virus antiserum neutralized the homologous and the Peiping viruses in about equal titer, while the St. Louis virus antiserum was able to neutralize only its homologous virus but not the Peiping virus. On the other hand, the Peiping virus antiserum was able to neutralize not only the homologus virus but also over 100 MLD of the Japanese B virus and partially 10 MLD of the St. Louis virus.

---

* These viruses originally obtained from Dr. Hashimoto and Dr. Webster, were kindly supplied to us by Dr. J. P. Wu, Division of Pediatrics, Peiping Union Medical College.

Conclusion. A virus (Peiping encephalitis virus) capable of producing encephalitis in Swiss mice and a monkey was isolated from a case of acute encephalitis. On the basis of animal pathogenicity and virus neutralization tests, the virus is considered to behave like the Japanese type B virus, but to be serologically distinguishable from the St. Louis encephalitis virus.

12081

Comparative Anticonvulsant Activity of 1-Diethylacetyl-5, 5-Cyclopentamethylene Biuret, Sodium Diphenyl Hydantoinate and Sodium Pentobarbital in Mice.*

Hamilton H. Anderson and S. Y. P'an.

From the Department of Pharmacology, Peiping Union Medical College, Peking, China.

The structural features which appear to account for the hypnotic action of the barbiturates are the presence of a –CONH– group[1] and an alkyl or acyl group replacing both hydrogen atoms in position 5.[2] Recently Hill and Degnan[1] have emphasized that the alkyl biurets have 2 –CONH– groups within their structure. They postulated that an acyl biuret containing branched chain alkyl groups might have hypnotic properties and low toxicity. Although Hesse and Taubmann[3] had previously reported on the pharmacology of certain biurets and some of their guanyl and thio derivatives, the use of these agents as hypnotics had not been proposed. They reported that guanyl-thiourea lowered the body temperature of rabbits in which artificial fever had been produced. The antipyretic action was believed to be due to increased heat loss and was abolished when animals were kept in a warm environment. The presence of the thio group seemed necessary for antipyretic action. When oxygen or an amino group was substituted for the thio group antipyretic action was lost but lowering of blood pressure occurred. Oral doses of the ethoxyphenyl derivatives of guanyl urea in 0.2 g amounts given 3 times a day to man were reported to relieve headache and other pains. Apparently analgesic action accompanied antipyretic effects.

---

* Sodium pentobarbital was supplied through the courtesy of Eli Lilly and Company, Indianapolis, Indiana.

[1] Hill, A. J., and Degnan, W. M., *J. Am. Chem. Soc.*, 1940, **62**, 1595.

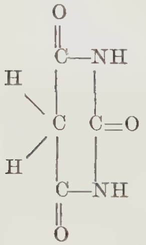
[2] Tatum, A. L., *Physiol. Rev.*, 1939, **19**, 472.

[3] Hesse, E., and Taubmann, G., *Arch. f. Exp. Path. u. Pharmakol.*, 1929, **146**, 113.

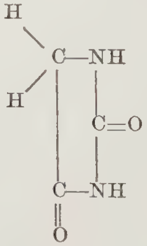
A series of acyl biurets synthesized by Hill and Degnan[1] indicated that these compounds compared favorably with sodium barbital in effectiveness as hypnotics. They reported that the biurets "do not show any anesthetic (depressor) action. The toxicities of these compounds, with the exception of those of the thiobiurets, are remarkably low; doses as high as 500 mg/kg have no permanent ill effects on the test animals. The presence of branched chains in the acyl radical increases effectiveness. Alkylation in the 5-position of the biuret chain also increases effectiveness, 1-diethylacetyl-5, 5-cyclopentamethylene biuret being the most active of the compounds tested."

Recently Ch'eng, Anderson and P'an[4] compared the hypnotic properties of this compound with those of paraldehyde and sodium barbital in white mice. Doses of 800 mg per kg of paraldehyde, 300 mg per kg of sodium barbital and 450 mg per kg of the 1-diethylacetyl-5, 5-cyclopentamethylene biuret were found to produce narcosis of longer duration with the dialkylacetyl biuret than with paraldehyde but of shorter duration than with sodium barbital. Dialkylacetyl biuret caused death in approximately half of the animals given 600 mg per kg intraperitoneally.

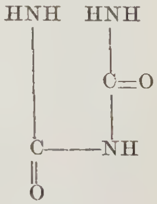
In view of the definite hypnotic activity of this dialkylacetyl biuret,† it was believed desirable to determine whether the compound was anticonvulsant as well. A comparison was made with sodium diphenyl hydantoinate (sodium dilantin) and sodium ethyl-1-methyl-butyl barbiturate (sodium pentobarbital). Dilantin, pentobarbital and biuret have analogous structural formulae of their molecules. Barbiturates are malonylurea derivatives while dilantin



Barbituric acid



Hydantoin



Biuret

---

[4] Ch'eng, C. H., Anderson, H. H., and P'an, S. Y., in press.

† Prepared by Dr. Charles H. Ch'eng, Yenching University, Peiping, China.

is a derivative of glycolyl urea and biuret is composed of 2 urea molecules linked together with the loss of one molecule of ammonia. In contrast to the other two, it is open chained. It will be noted that all of these agents have –CONH– groups in their molecules.

Putnam and Merritt[5, 6] have reported the anticonvulsant properties of dilantin and this agent has proved to be useful in the treatment of epilepsy. Pentobarbital has been shown by Swanson and others to be effective in antidoting cocaine and strychnine poisoning.[7, 8, 9] The antagonism between picrotoxin and pentobarbital has also been demonstrated by Koppanyi, *et al.*[10]

Convulsants which act on different levels of the central nervous

TABLE I.
Comparative Anticonvulsant Activity of Dialkylacetyl Biuret, Sodium Dilantin and Sodium Pentobarbital in Mice.

Convulsant		Hypnotic					
Name	Dose= LD_{100} mg/kg	Name	Dose= LD_{50} mg/kg	No. of animals	Avg time of death, min.	No. having convulsions	No. dying
Cocaine*	150‡	—	—	10	55	10	10
”	”	Alkyl biuret	600§	12	207	3	12
”	”	Sodium dilantin	200[13]	6	194	6	6
”	”	Na pentobarbital	150[14]	6	7	2	6
Picrotoxin	9.75[11]	—	—	10	10	5	10
”	”	Alkyl biuret	600	10	—	0	0
”	”	Sodium dilantin	200	5	19	5	5
”	”	Na pentobarbital	150	10	12	0	5
Strychnine	2[12]	—	—	10	6	5	10
”	”	Alkyl biuret	600	10	66	0	1
”	”	Sodium dilantin	200	10	1560	8	5
”	”	Na pentobarbital	150	5	13	1	5

*Larger doses were required to kill all animals, but no animal given this amount plus an hypnotic survived.

‡ Determined in this laboratory.

§ Determined in this laboratory.

---

[5] Putnam, T. J., and Merritt, H. H., *Science*, 1937, **85**, 525.

[6] Merritt, H. H., and Putnam, T. J., *Arch. Neurol. and Psych.*, 1938, **39**, 1003; *J. A. M. A.*, 1938, **111**, 1068; *Arch. Neurol. and Psych.*, 1939, **42**, 1053.

[7] Swanson, E. E., and Shonle, H. A., *J. Lab. and Clin. Med.*, 1931, **16**, 1056.

[8] Barlow, O. W., *J. A. M. A.*, 1932, **98**, 1980.

[9] Swanson, E. E., *J. Am. Pharm. Assn.*, 1935, **24**, 959.

[10] Koppanyi, T., Linegar, C. R., and Dille, J. M., *J. Pharm. and Exp. Therap.*, 1936, **58**, 199.

[11] Chakravarti, M., *J. Pharm. and Exp. Therap.*, 1939, **67**, 154.

[12] Schwartze, E. W., *J. Pharm. and Exp. Therap.*, 1922, **19**, 273.

[13] Gruber, C. M., Haury, V. G., and Drake, M. E., *J. Pharm. and Exp. Therap.*, 1940, **68**, 433.

[14] Gates, W. H., *Science*, 1932, **76**, 349.

system were chosen for study. These were cocaine, picrotoxin and strychnine. Except with cocaine, single doses equivalent to the LD_{100} (a surely fatal dose) for each convulsant were given subcutaneously to inbred white mice. Immediately before or after this injection the hypnotic was given intraperitoneally. Single or repeated doses equivalent to one or more LD_{50}s were administered. There were 24 or more animals in each treated group. Thirty mice served as controls and were given the convulsants alone. Animals were observed for a period of 48 hours and mice that lived longer were considered as "survived".

Table I summarizes the results of this study. Convulsions developed early and persisted until death in 5 animals which had been injected with strychnine and treated with dilantin and in 3 of the other 5 mice which survived this treatment. Administration of an hypnotic either before or after the convulsant was given did not alter the protective action significantly. While none of the hypnotics antagonized cocaine completely, the dialkylacetyl biuret and pentobarbital usually antagonized the convulsant action of cocaine. This result agrees with the findings of Dragstedt and Lang[15] who found that barbital was effective in preventing or relieving cocaine convulsions but it did not decrease the mortality percentage in rabbits.

Under the conditions of this experiment the biuret was superior to pentobarbital or dilantin in antagonizing and detoxifying the action of lethal doses of picrotoxin and strychnine. In view of the frequent occurrence of toxic side-actions in epileptic patients treated with sodium dilantin, even under careful supervision and adjustment of dosage[16, 17] the anticonvulsant activity of the dialkylacetyl biurets and their derivatives certainly deserves further study.

Summary. The anticonvulsant activity of 1-diethylacetyl-5, 5-cyclopentamethylene biuret was compared in inbred mice with that of sodium dilantin and sodium pentobarbital. The biuret and pentobarbital protected the majority of animals given cocaine but they did not prevent death. All cocaine-treated mice given dilantin died from convulsions. Against surely fatal doses of picrotoxin and strychnine the dialkylacetyl biuret was effective in 19 of 20 animals. Dilantin had no effect against picrotoxin but saved half of the strychnine-treated animals. Sodium pentobarbital prevented convulsions in mice given picrotoxin and saved 5 of 10. It was anticonvulsant in the majority of strychnine poisoned mice but did not prevent death.

---

[15] Dragstedt, C. A., and Lang, V. F., *J. Pharm. and Exp. Therap.*, 1928, **32**, 215.

[16] Fetterman, J. L., *J. A. M. A.*, 1940, **114**, 396.

[17] Lennox, W. G., *J. A. M. A.*, 1940, **114**, 1351.

12082

Estimation of Biological Activities of Purified Tuberculin by the Hopkins Tube.

Sam C. Wong and George Ouyang.

From the Departments of Bacteriology and Immunology and Public Health, Peiping Union Medical College, Peiping, China.

Since the protein of *Mycobacterium tuberculosis* is now considered to be the active principle responsible for the allergic reaction in the Mantoux intracutaneous test, the dry weight of this substance has been employed as a basis of measuring biological activities on the tacit assumption that all preparations by the same method yield products of the same potency. Recent findings[1, 2] including observations of our own[3] have shown that such a belief is no longer tenable. Indeed, under certain conditions[4] the activity of the substance may be largely destroyed without any material alteration in its dry weight. Since the conditions responsible for these fluctuations in potency are not well understood it may seem obvious that every preparation requires careful standardization. The only satisfactory method for this purpose, according to Seibert and DuFour,[2] is the sensitive human skin, although sensitive guinea pigs may be used for preliminary titrations. Even the human skin does not always remain uniform in its reaction to tuberculin. For example we found[3] that little or no difference could be detected among the following strengths of purified tuberculin when comparative Mantoux tests were performed on 15 human subjects: 0.000,04, 0.000,02, 0.000,01 mg. Similar results were also found by Seibert and DuFour.[2] Apart from this limitation of the tissue-reaction, the expense, time, and technical difficulties involved would seem to warrant a search for a more practical method. In the present communication an attempt was made to determine whether a precipitative method would fulfill the requirements.

Methods and Material. Purified tuberculins of various strength were employed for the present study. The details of the preparation will be reported elsewhere. In addition, a PPD prepared by a new method[5] and kindly supplied by Dr. Seibert was used as the stand-

---

[1] Jensen, K. A., Bindslev, G., Moller, S., Hansen, A., and Lind, P., *Tubercle,* 1938, **19**, 386.

[2] Seibert, F. B., and DuFour, E. H., *Am. Rev. Tuberc.,* 1940, **41**, 471.

[3] Unpublished results.

[4] Wong, Sam C., and Ouyang, G., Proc. Soc. Exp. Biol. and Med., in press.

[5] Seibert, F. B., *J. Biol. Chem.,* 1940, **133**, 593.

ard. Comparisons of biological activities were done on human subjects employing the same precautions, the dosage of tuberculin, the interval of reading, and the site of injections previously emphasized.[4] The volume of precipitate in a given solution was measured in the Hopkins tube as follows: One cc of a clear solution of purified tuberculin was delivered with a volumetric pipet into a Hopkins tube, an equal volume of 25% trichloracetic acid was added, the mixture was refrigerated overnight, and then centrifuged at 2300 r.p.m. for 30 minutes. A preliminary reading was first taken and the precipitate was tightly packed with a stiff wire. The centrifugation was repeated to ensure that a constant reading has been obtained. It was found that one centrifugation usually suffices. A duplicate sample was always run and the average results of the readings were taken as the value. However, if the difference in readings was greater than 0.002 cc, the whole procedure was repeated employing fresh samples. It is to be especially noted that all solutions employed for this test must be perfectly clear.

Relation Between Weight and Volume. In the first experiment we attempted to correlate the dry weight of various preparations of purified tuberculin and the volume of precipitate. For this purpose we employed 4 different batches of our tuberculin together with Seibert's PPD. The nitrogen-contents of the former preparations were equal, 13.27% being found, while that of the latter, according to Seibert,[5] was 16.3%. The results are presented in Table I.

All readings except those of PPD were the average of 4 different determinations. In case of the latter only duplicate samples were run because of lack of material. From the data it is clear that a definite relation exists between weight and volume of the same preparation and that volumes of precipitate vary with different preparations when similar weights are compared.

Relation Between Biological Activity and Volume. Since the dry weight of purified tuberculin can not be employed as an absolute measurement of Mantoux-activity as emphasized elsewhere in the report and since the volume of precipitate varies with different preparations of purified tuberculin it is of interest to determine

TABLE I.
Relation Between Weight and Volume of Precipitate of Various Tuberculins.

	Hopkins tube reading in cc				
Wt in mg	Lot A	Lot B	Lot C	Lot D	PPD
5	.020	.020	.010	.010	.023
2½	.011	.010	.005	.005	.013
1	.004	.004	.003	.002	.005

whether there exists a relation between activity and volume. It was found by careful titrations in the human skin that the various tuberculins presented in Table I possessed the following relative potencies: Lots A and B were equal, lots C and D were half as strong as lot A, and PPD was the strongest. When these values are compared with the volumes of precipitate given in Table I, two points may be emphasized: In the first place tuberculins of the same activity, such as lots A and B gave similar volumes of precipitate while those of different activities yielded different values. In the second place lots C and D were found to be half as strong as lots A and B, their volumes of precipitate were only half of that of the latter. Similarly, PPD was stronger than lot A, its value was greater than the latter. In the light of these findings it should be possible to adjust other preparations of purified tuberculin to Seibert's standard employing the following equation:

$$\frac{\text{Reading in Hopkins tube} \times \text{Constant}}{\text{cc taken}} = (\text{n}) \text{ mg per cc}$$

n being the value of the activity of a given solution of purified tuberculin in terms of PPD. Since the volume of precipitate and the weight of PPD are known the same equation may be applied to compute the value of the constant, the average of which is 203.

Application. In order to demonstrate the usefulness of the above equation we proceeded to compute the biological activity of our tuberculin, lot A, in terms of PPD, employing the Hopkins-tube values recorded in Table I. Thus a 1% solution when computed

TABLE II.
Showing Size of Reaction.

	Reading in mm	
Subject	PPD (Seibert's)	Lot A
CKM	20 x 20	20 x 20
LCT	17 x 20	20 x 20
LIH	23 x 23	24 x 25
LLC	16 x 20	18 x 20
SS	25 x 25	25 x 25
LPS	15 x 15	15 x 15
PKL	20 x 20	20 x 20
TTC	25 x 25	25 x 27
WCS	15 x 16	15 x 15
YMS	10 x 10	10 x 10
LKS	15 x 15	15 x 15
CKP	20 x 20	20 x 20
KCL	0 x 0	0 x 0
LPH	0 x 0	0 x 0
WTC	0 x 0	0 x 0

was found to be only 0.8%. When this solution was diluted according to the latter value and compared with Seibert's standard in human subjects complete agreement of Mantoux activities resulted. The data shown in Table II are self-explanatory.

In a similar manner we have applied the equation for estimating the biological activities of a large number of preparations. These included solutions of known concentration but of different activities and solutions prepared by dissolving at random tuberculin-powder in buffer solution. The results of these findings showed consistent agreement of biological activities between Seibert's PPD and our various preparations of purified tuberculin. Incidentally it should be emphasized that the equation employing the constant 203 is not applicable under the following conditions: unpurified heated or undenatured tuberculins, and purified tuberculins hydrolyzed by alkali. The most accurate reading lies between 0.01 to 0.03 cc, while the least is below 0.005 cc. In the former an error of 20% may occur, while in the latter about 50%.

Comment. The Hopkins-tube method described above is not new, Seibert[6] has made use of the same in the standardization of synthetic medium tuberculin. It has not, however, been applied in correlating biological activity of various preparations of purified tuberculin. Why tuberculins of the same weight and the same nitrogen content but differing in biological activities yielded different volumes of precipitate is not clear. The possibility that a part of the tuberculin of the less active preparation is not precipitated by the trichloracetic acid is rendered unlikely by the fact that the original tuberculin represents that portion precipitated by the acid. It seems plausible, however, that under the conditions of the experiment less active preparation gave a more compact precipitate than the active preparations.

Since the method has been applied to purified tuberculins prepared from only one strain of *Mycobacterium tuberculosis* (H37), we believe it is premature to suggest its use for the standardization of all purified tuberculins. Still we believe that the results so far shown indicate that such a method is useful for preliminary estimation of tuberculin of unknown activity or of tuberculin whose weight is not known.

Conclusion. On the basis of the above results and subjected to the limitations mentioned it is justifiable to conclude that the Hopkins-tube method is useful for the estimation of biological activity of various preparations of purified tuberculin.

---

[6] Seibert, F. B., *J. Biol. Chem.*, 1928, **78**, 345.

12083

Effect *in Vitro* of Curare and Beta-Erythroidin Hydrochloride on Choline Esterase of Human Blood Serum. I.*

MEYER M. HARRIS AND RUTH S. HARRIS.

From the Department of Internal Medicine, New York State Psychiatric Institute and Hospital, New York City.

Because of the similarity of some preparations of curare and beta-erythroidin in producing paralysis of the skeletal musculature both have been used in man for the relief of some non-voluntary muscular rigidities and spasms due to involvement of the nervous system[1, 2] and more recently for the prevention of fractures resulting from metrazol convulsant therapy in mental patients.[3, 4, 5] Some investigators[4] have found that it required about 10 to 20 times the dose of beta-erythroidin hydrochloride to that of some curare preparations to produce similar states of paralysis in man for the purpose of preventing fractures in metrazol convulsant therapy.

Owing to these differences in potency in producing paralysis, and the rôle of acetylcholine[6, 7] in the mechanism of muscular contraction, it was planned to investigate the effect of these substances on the choline esterase activity of human serum.[8]

Procedure. The choline esterase activity of the serum was determined by the continuous titration method as described by Hall and Lucas,[9] using acetylcholine bromide or acetylcholine iodide as the substrate. The amount of acetic acid liberated in 20 minutes by the choline esterase activity of 0.5 ml of serum, expressed in terms of the milliliters of N/100 sodium hydroxide, required for its neutralization, was used as the measure of the enzymatic activity.

---

* A preparation of curare under the trade mark of "Intocostrin" was supplied for experimental purposes by E. R. Squibb & Sons, New York City. A sample of beta-erythroidin hydrochloride used in this study was obtained from Dr. D. Ewen Cameron. This drug is a purified isomer of erythroidin-hydrochloride prepared by Merck & Company of Rahway, N.J., and made available by them for clinical investigation.

[1] West, R., *Proc. Roy. Soc. Med.*, 1935, **28**, 565.

[2] Burman, M. S., *Arch. Neur. and Psychiat.*, 1939, **41**, 307.

[3] Bennet, A. E., *J. Am. Med. Assn.*, 1940, **114**, 322.

[4] Rosen, G. R., Cameron, D. E., and Ziegler, J. B., *Psychiat. Quart.*, 1940, **14**, 3.

[5] Gray, R. W., Spradling, F. L., and Fechner, A. H., *Psychiat. Quart.*, 1941, **15**, 159.

[6] Brown, G. L., *Physiol. Rev.*, 1937, **17**, 485.

[7] Eccles, J. C., *Physiol. Rev.*, 1937, **17**, 538.

[8] Ammon, R., and Voss, G., *Pflüg. Arch. f. d. ges. Physiol.*, 1934-5, **235**, 393.

[9] Hall, G. E., and Lucas, C. C., *J. Pharm. and Exp. Therap.*, 1937, **59**, 34.

Corrections were made as recommended by Hall and Lucas. Burette readings taken every 5 minutes over the 20-minute period of enzymatic hydrolysis and plotted against time gave a straight line as indicated by the above authors. The stock solution of curare ("Intocostrin") was equivalent in physiological activity to 10 mg of crude curare per ml. The milligrams of curare indicated in the tables were calculated on this basis. Various dilutions of the curare were made with boiled distilled water. The beta-erythroidin was obtained in dry crystalline form and was dissolved in boiled, distilled water in the various amounts used in the experiments. The total volume containing all the constituents was always kept equal to that described in the method of Hall and Lucas. Blanks containing all the constituents, except the serum were always run at the same time and were used to correct for spontaneous decomposition of the acetylcholine.

In one experiment the effect of the simultaneous addition of curare and beta-erythroidin was investigated.

Observations. The results of typical experiments are recorded in Tables I, II, and III.

Discussion. It will be seen from the tables that very minute amounts of curare produced a marked inhibition of choline esterase activity. The degree of inhibition, up to about 60%, was approxi-

TABLE I.
Effect of Curare on Choline Esterase Activity of Human Serum.

Curare added mg	Choline esterase activity, of 0.5 ml serum, units*	Decreased choline esterase activity, %
0.0	3.11	0
0.016	0.46	85.2
0.004	1.19	61.7
0.002	2.01	35.4
0.001	2.56	17.7

TABLE II.
Effect of Beta-Erythroidin Hydrochloride upon Choline Esterase Activity of Serum.

β-Erythroidin HCl added mg	Choline esterase activity of 0.5 cc serum, units*	Decreased choline esterase activity, %
0	3.9	0
0.16	3.9	0
0.84	3.9	0
50.0	3.9	0

TABLE III.
Effect of Adding Curare and Beta-Erythroidin to Same Mixture upon Choline Esterase Activity of the Serum.

Curare added mg	β-Erythroidin HCl added, mg	Choline esterase activity of 0.5 cc serum, units*	Decreased Choline esterase activity, %
0.0	0.0	3.75	0
0.004	0.0	1.45	61.3
0.0	0.16	3.85	0
0.004	0.16	1.54	58.9

*Units are equivalent to the numbers of ml of N/100 NaOH required to neutralize the acetic acid liberated from the acetylcholine. The figures have been corrected as indicated in the text.

mately proportional to the amount of curare added. The maximum inhibition of esterase activity obtained was 85%. (Table I.)

In contrast, the addition of beta-erythroidin hydrochloride, in amount up to 3000 times that of the largest amount of curare, had no effect upon the choline esterase activity. (Table II.) The combined addition of curare and beta-erythroidin hydrochloride produced the same inhibition as that of an equal amount of curare alone. (Table III.)

The important rôle attributed to acetylcholine and choline esterase in the physiology of muscle and the nervous system[6, 7] should lead one to expect that 2 substances which differ so markedly in their effect upon this enzyme system would also differ markedly in physiological action. The production of paralysis of the muscular system by both substances is probably only a gross similarity of action. This view finds substantiation in a number of physiological experiments with curare and curare-like substances and their antidotes in warm and cold blooded animals. The fact that eserin has been used as an antidote for curare and the explanation offered by some that eserin produces its effect by the inhibition of the choline esterase would appear to be rather questionable in view of the marked inhibition produced by curare alone. That other mechanisms of action may be involved seems likely from the findings of other investigators.[10-14] Further knowledge regarding the differences in the mode of action of these substances may throw additional light upon neurophysiological

---

[10] Ing, H. R., *Physiol. Rev.*, 1936, **16**, 527.

[11] Jacobsohn, D., and Kahlson, G., *Skand. Arch. f. Physiol.*, 1938, **79**, 26.

[12] Roepke, M. H., *J. Pharm. and Exp. Therap.*, 1937, **59**, 264.

[13] Pusitz, M. E., Lattimore, J. L., Gold, A., and Ebendorf, H., *J. Kansas Med. Soc.*, 1940, **41**, 374.

[14] Rosenbluth, A., Lindsley, D. B., and Morison, R. S., *Am. J. Physiol.*, 1936, **115**, 53.

mechanisms and also help to explain certain differences in the therapeutic action of these substances. Such information should be of value in the clinical use of these substances.

Since the curare used is not a pure chemical substance but represents a mixture of various alkaloids,[15] it will be important to study some of the pure alkaloids such as curarine and curine and other curare constituents regarding their effect upon choline esterase in order to determine which substance or substances in the curare produce the marked inhibition of the choline esterase activity and whether these parallel the paralytic action or toxicity of the curare. Plans are under way for an investigation of this phase of the problem.

The finding, as previously indicated, that within a certain range the degree of inhibition of choline esterase activity is proportional to the amount of curare added to the experimental mixture, may afford an *in vitro* method for assaying curare preparations as far as the constituent inhibiting choline esterase is concerned. Such determinations may help to explain some of the differences obtained clinically with different curare preparations. This requires further study.

Summary and Conclusions. 1. *In vitro* experiments have been carried out regarding the effect of curare and beta-erythroidin hydrochloride upon the activity of choline esterase of human serum. 2. Very small amounts of curare had a marked inhibiting effect upon the choline esterase activity of human serum. 3. The degree of inhibition of enzyme activity up to 60% was approximately proportional to the amount of curare added. The possible application of this finding for assaying curare preparations is indicated. 4. Beta-erythroidin hydrochloride in amounts 3000 times as large as that of the largest amount of curare had no effect upon the choline esterase activity of the serum. 5. The possible importance of the marked difference in effect between curare and beta-erythroidin hydrochloride upon choline esterase activity is discussed. 6. Plans for further investigation are indicated.

---

[15] Henry, T. A., *The Plant Alkaloids*, P. Blakiston's Sons, Philadelphia, Pa., 1939.

12084

Effect in Man of Curare and Metrazol Administration on Choline Esterase Activity of Blood Serum. II.*†

Meyer M. Harris and Ruth S. Harris.

From the Department of Internal Medicine, New York State Psychiatric Institute and Hospital, New York City.

In a previous publication[1] we reported that small amounts of curare *in vitro* inhibit the choline esterase activity of human serum, the extent of the inhibition varying with the amount of curare added. In this paper we wish to report upon the effect of the intravenous injection of curare‡ in man upon the choline esterase activity of the serum and the duration of this effect.§ It was hoped that such determinations would yield information as to the relation of the induced muscular paralysis to the choline esterase activity and also afford a means of approximating the amount of the drug present in the blood stream at various periods after its administration. Information regarding the latter might be of value in studies on the therapeutic use of the drug in various neuromuscular conditions.

A review of the literature revealed no studies of this nature carried out in man. Felger and Kowarzyk[2] reported that the intravenous injection of curare in the rabbit had, contrary to their

---

* We wish to acknowledge the assistance of Dr. Bernard L. Pacella and Dr. William A. Horwitz in the administration of the medication.

† Squibb & Sons supplied their curare preparation known under the trade name of "Intocostrin" for these studies. Each milliliter of this curare solution was equivalent to the physiological activity of 10 mg of the crude drug.

[1] Harris, M. M., and Harris, R. S., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 619.

‡ The blood was obtained from a group of mental patients who were receiving curare as a preventive measure against fractures during metrazol convulsant therapy and also from a neurological patient, W.M., who was suffering from coarse tremors of the upper and lower extremities.

§ One must keep in mind the fact that curare is not a pure chemical substance but a mixture and it is possible that some of the substances present may be physiologically active without affecting the enzymatic activity. As indicated in the previous publication it is planned to investigate this phase of the subject. Unpublished experiments carried out by us indicate that the incubation of either serum or defibrinated blood at 37.5°C for 1 hr with curare does not alter the inhibiting power of the curare on the choline esterase activity of the serum. The red blood cells of the defibrinated blood do not appear to take up any measurable amount of the material in the curare which inhibits choline esterase activity.

[2] Felger, J., and Kowarzyk, H., *Compt. Rend. Soc. de Biol.*, 1938, **127**, 1149.

expectations, an inhibiting effect upon the choline esterase activity of the blood. They stated, however, that the curare first produced blocking of the neuromuscular junction without affecting the choline esterase activity of the blood and only subsequently was the latter affected. No detailed experimental data were given in their paper.

Procedure. Samples of blood were withdrawn from the cubital vein of the arm both before and at various intervals after medication. Compression was applied only for a short interval to facilitate venipuncture. Care was taken to avoid hemolysis. The blood was allowed to clot and the serum was removed in about one-half to one hour after the venipuncture. The curare solution was injected intravenously at the rate of one milliliter per 30 to 45 seconds until the desired degree of muscular paralysis was produced. This usually required from 7 to 10 ml of the curare solution. In 2 cases no other medication was given and blood samples were taken at several intervals up to 30 minutes after the injection as shown in Table I. In 4 cases metrazol was injected intravenously following the injection of curare in order to produce convulsions. Blood samples were collected at various intervals up to 6 hours from the time of completion of the injection of curare as indicated in Table II.

The choline esterase activity of each sample of serum was determined as described in a previous publication[1] by the continuous titration method as adapted by Hall and Lucas.[3] The number of milliliters of N/100 NaOH required to neutralize the acetic acid

TABLE I.
Effect of Intravenous Injection of Curare on Choline Esterase Activity of Serum.

Patient	Wt lbs	Time after curare injection*	Choline esterase activity of 0.5 ml of serum, units	Decreased choline esterase activity, %	Remarks
(S.S.) (F)	124	Before	3.18	0	8 ml curare sol. inj. in 6 min.
		6 min.	1.13	64.5	
		18 ”	1.44	54.7	
		30 ”	1.55	51.3	
(W.M.) (M)		Before	2.90	0	10 ml curare sol. inj. in 7 min.
		Immed. after	1.25	57.0	
		15 min.	1.68	42.7	
		30 ”	2.11	27.3	

*Time indicated is that which elapsed between the completion of the injection of curare and the obtaining of the particular blood specimen.
M—Male. F—Female.

---

[3] Hall, G. E., and Lucas, C. C., *J. Pharm. and Exp. Therap.*, 1937, **59**, 34.

TABLE II.
Effect of Intravenous Injection of Curare and Metrazol on Choline Esterase Activity of the Serum.

Patient	Wt lbs	Time after curare injection*	Choline esterase activity of 0.5 ml of serum, units	Decreased choline esterase activity, %	Remarks
(A.F.)	135	Before	3.66	0	9.6 ml curare sol. inj. in 7 min. followed immediately by 7 ml of metrazol. Convulsion produced.
(M)		11 min.	1.68	54.1	
		5 hr. 8 min.	3.01	17.8	
(V.T.)	156	Before	4.63	0	9.5 ml curare inj. in 7 min. followed immediately by 6 ml of metrazol and 8 ml 5 min. later. Convulsion produced by second dose of metrazol.
(M)		24 min.	2.22	52.0	
		5 hr. 25 min.	3.35	27.6	
(V.D.)	108	Before	3.23	0	7 ml curare inj. in 4½ min. followed by 6 ml of metrazol. Convulsion produced.
(F)		Immediately after curare	0.95†	71.0†	
		6hr	2.84	12.1	
(B.S.)	120	Before	4.40	0	8 ml curare in 7 min. followed by 5 ml of metrazol. Convulsion produced.
(F)		6 min.	1.28†	68.3†	
		6 hr.	2.71	39.4	

*The time indicated is that which elapsed between the completion of the injection of curare and the obtaining of the blood specimen.

†These second blood specimens were obtained just before the metrazol was injected in cases (V.D.) and (B.S.). The second specimen was taken after metrazol had also been injected in cases (A.F.) and (V.T.).

liberated from acetylcholine in 20 minutes at 37.5°C at pH 8.0 by the choline esterase present in 0.5 ml of serum and corrected according to the recommendations of Hall and Lucas,[3] is reported as so many units of enzyme activity.

Observations. It will be seen from Table I and patients (V.D.) and (B.S.) in Table II, that immediately or within a few minutes after the injection of curare there is a marked decrease in choline esterase activity ranging from 57% to 71% of the initial values. In patients (A.F.) and (V.T.) (Table II) the second specimens of blood were taken after metrazol also had been injected and the patients had had a convulsion. The intervals which had elapsed between the completion of the injection of curare and the taking of the second blood samples were 11 minutes and 24 minutes and the decrease in choline esterase 54.1% and 52.0% respectively. In the subsequent intervals the choline esterase activity in each case tended to rise toward its initial value at variable rates. These rates of recovery were independent of the size of the dose of curare or the weight of the patient. (Tables I and II.) In the cases which received metrazol in addition to the curare (Table II) the rate of recovery from inhibition of choline esterase activity appeared to bear no relation to the dose of metrazol which was administered.

It is of interest to note that, even after 5 to 6 hours the choline esterase activity was still from 12.1% to 39.4% below the respective initial values.

It may be of interest to point out at this time that 30 minutes after the injection of curare the patients showed no evidence of paresis although some patients stated that their muscles felt tired.

Discussion. It would be beyond the scope and purpose of this paper to enter upon any extensive discussion of the various phases of physiology and pharmacology pertaining to the rôle of acetylcholine and choline esterase and the action of various antidotes to curare. Some references to this phase of the subject have been made in the previous paper and also have been considered to some extent in a number of reviews.[1, 3, 4] It may be of interest to mention, however, in view of the marked inhibition of choline esterase activity that Pusitz[5] and his coworkers indicate that the injection of mecholyl tended to aggravate rather than relieve curare intoxication.

Our findings in the human are in agreement with the findings of Felger and Kowarzyk[2] in the rabbit that the injection of curare produces an inhibition of the choline esterase activity of the blood. However, our finding that marked inhibition of choline esterase activity is still present when muscular paralysis has disappeared would appear to differ from their findings in the rabbit.

The degree of inhibition of choline esterase activity produced by the intravenous injection of curare is of the same order of magnitude as that obtained in the *in vitro* experiments reported in our previous publication.[1] It, therefore, should be possible from the amount of inhibition of choline esterase activity to estimate the amount of the inhibiting portion of the curare present in the blood stream at any period after injection by carrying out preliminary *in vitro* experiments with serum or blood from the experimental subject as described in our previous publication.[1] It also should afford a tool for the investigation of clinical, physiological and pharmacological problems pertaining to curare.

---

[4] Ing, H. R., *Physiol. Rev.*, 1936, **16**, 527.

[5] Pusitz, M. E., Lattimore, J. L., Gold, A., and Ebendorf, H., *J. Kansas Med. Soc.*, 1940, **41**, 374.

Summary and Conclusions. 1. The intravenous injection of curare produces a marked inhibition of the choline esterase of human serum. 2. The inhibition of this esterase activity gradually diminishes at a variable rate in different patients, but is still present 5 to 6 hours after the injection of curare. 3. The degree of inhibition of the esterase activity was similar to that obtained in *in vitro* experiments previously reported. 4. A method is indicated for estimating the amount of the choline esterase inhibiting constituent of curare in the blood by the use of its inhibiting effect on esterase activity. Some of the possible applications of the method are pointed out. 5. The relation between the effect of curare on choline esterase and the production of muscular paralysis is discussed.

12085 P

Strychnine Tetanus and the Mechanism of Neuronal Synchronization.*

F. BREMER. (Introduced by J. F. Fulton.)

From the Laboratoire de Pathologie Generale de l'Universite Libre de Bruxelles.

Oscillographic study indicates that spinal strychnine tetanus is the most perfect expression of the synergy of elementary electrical activities in a mass of nerve cells. Strychnine tetanus was studied in the curarized cat under light dial anesthesia (10 to 20 mg per kg) with the special purpose of analyzing the mechanism of neuronal synchronization. The following facts were demonstrated:

1. Tetanic waves, recorded from any segmental level of the spinal cord by means of an active electrode thrust into the grey matter of the anterior horn, have regularity of form, amplitude and rhythm of an alternating current. Their frequency varies from 30 to 10 per second, their voltage from 500 to 100 microvolts. Usually there is an inverse relationship between voltage and frequency. This relationship becomes most apparent when the frequency of the tetanic waves has been increased as the result of an intravenous injection of nicotine.

2. With each tetanic wave there is a corresponding strong discharge of centrifugal action potentials in the anterior roots. This

---

* This work was completed in May, 1940, but because of war conditions its publication has been delayed until now.

discharge begins with the rising phase of the tetanic wave and is maximal at its peak.

3. Cell groups of the posterior horns do not participate in the strychnine tetanus. They are likewise refractory to the synchronizing action of other agents.

4. Waves simultaneously recorded from cervical, dorsal and lumbosacral regions of the cord are practically synchronous. They are never out of phase by more than 3 milliseconds.

5. This synergy cannot be explained by synaptic transmission of intraspinal impulses from a focus playing the rôle of a pacemaker, for the following reasons: (a) The cervical and lumbar reflex waves produced by stimulation of a posterior lumbar root (break shocks) are separated by a time interval which is never less than 8 milliseconds even if the spinal cord is strongly strychninized with a just subtetanic dose. (b) Strychnine tetanus is abolished by depressor agents (ether anesthesia for 30 seconds; anelectrotonus) at doses or intensities which do not affect, or only slightly diminish the amplitudes of spinal reflex waves. Conversely, a strong tetanus may continue in the absence of all electrically demonstrable reflex activity in the intervals between tetanic discharges. (c) Synchronization of the tetanic waves produced by two adjacent spinal segments may persist after complete separation of the segments by spinal transection.

6. During the short phase of a few seconds which follows the injection of the tetanizing dose and immediately precedes onset of the tetanus, reflexogenous stimuli (break shocks) produce synchronous lumbar and cervical waves which by their form and by the constancy of their amplitude can be recognized as evoked tetanic waves. The onset of the tetanus is characterized by the appearance of spontaneous waves, which are all similar and occur in increasing numbers during the intervals between the evoked waves.

From these experimental data one may conclude that the mechanism producing synchronism of electrical pulsations throughout the entire ventral spinal grey matter is independent of all synaptic transmission of impulses. Everything suggests that this neuronal synergy is effected by an intercellular action, truly electrical in nature, which spreads from an occasional focus with the speed of an explosion and synchronizes, over wide areas, the pulsations of autorhythmic elements possessing nearly identical individual frequencies.

12086 P

Vasopressin Secretion by Cells of Hypophyseal Floor of Diencephalon Shown by Transplantation.*

Raymond F. Blount.

From the Department of Anatomy, University of Minnesota.

Since it is well established that vasopressin is produced by the neural portion of the hypophysis it is of interest to know when secretion starts and by what cells it is first produced. The derivatives of the infundibular process in the amphibian may be divided into the saccular wall, the lateral processes, the hypophyseal floor and the pars nervosa proper. Hormone production is usually attributed either to nerve endings or to pituicytes. It has been pointed out that secretion in tissue cultures of the posterior lobe by Geiling and Lewis[1] can not be attributed to nerve endings since they would have degenerated in such cultures. Similarly, nerve endings are eliminated in transplantations, which we[2] have made of the hypophysis in urodele embryos for they often do not have specific nerve connections but nevertheless secrete vasopressin.

The present experiments deal with the transplantation of the epithelial rudiment of the hypophysis together with the adjacent floor of the diencephalon but with no other components of the brain. For this work embryos of *Ambystoma maculatum* have been used at about Harrison's stage 31. The grafts were placed above and posterior to the pronephros in hosts of the same age. These transplants give rise to a vesicular pars sacculosa and the hypophyseal floor adjacent to the pars intermedia of the epithelial portion. These experiments and earlier ones[2] show that the vasopressin first appears about stage 43. This is indicated by vasoconstriction in animals carrying additional hypophyses as transplants and by vasodilation in hypophysectomized animals. Later, at about stage 45, correlated growth differences appear in which the gills are short and the tail fin narrow.[3] At these early stages there is, in the normal animal, no neuropilum beneath the hypophyseal floor and the pars nervosa proper has not yet developed. A similar situation is present in the transplants above described. Certainly it is unlikely that the

---

* This work has been aided by the Graduate School Research Fund of the University of Minnesota.

[1] Geiling, E. M. K., and Lewis, M. R., *Am. J. Physiol.*, 1935, **113**, 534.

[2] Blount, R. F., *J. Exp. Zool.*, 1935, **71**, 421.

[3] Blount, R. F., *J. Exp. Zool.*, 1935, **70**, 131.

squamous ependyma of the sacular wall of the infundibulum is secreting hormones. This places the responsibility for vasopressin secretion at this age upon the cells of the hypophyseal floor which are columnar and have considerable cytoplasm.

It is interesting that pituicytes, most frequently given the function of secretion, are thought to be derived from the ependyma as is demonstrated in the bird by Griffiths.[4] It is also interesting that the hypophyseal floor is essentially a modified ependyma. In mid-larval life, both in the normal hypophysis and in functional transplants, the processes of the cells of the hypophyseal floor can be seen. Certain cells appear to be detaching themselves and may be developing pituicytes. Further experimental and cytological studies are in progress upon the development and function of this region.

12087

Bacteriostatic Properties of Histiocytes Toward *Mycobacterium tuberculosis* as Determined by the Single Cell Method.*†

MORTON C. KAHN.

From the Department of Public Health and Preventive Medicine, Cornell University Medical College, New York City.

The fate of *Mycobacterium tuberculosis* within the phagocytic cell has been a subject of great interest to numerous investigators. Cunningham, Sabin and their coworkers[1] believed that the monocyte and clasmatocyte differed in structure, the former becoming changed into the epithelioid cell and in view of its inability to destroy the tubercle bacilli, the organism lived as a parasite within the transformed cell. Sabin and Doan[2] found clasmatocytes from tuberculous rabbits filled with acid-fast debris and concluded that this organism is as freely fragmented by the clasmatocyte as are other ingested particles.

---

[4] Griffiths, M., *Endocrinology*, 1940, **26**, 1032.

* The author wishes to express his deep appreciation for the technical assistance of Ann Sonnentheil, Fanny Bach Parsons, Helen Schwarzkopf, Helen Swann and Helen Kellogg.

† This study is part of a group investigation being conducted in conjunction with the Medical Research Committee of the National Tuberculosis Association.

[1] Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 231.

[2] Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, **46**, 627.

Borquist and Rowe[3] found no evidence to indicate fragmentation of the organisms by the cell even though some of the mycobacteria had been ingested for many days. They suggested that the longevity of the epithelioid cell rather than the multiplication of the mycobacterium therein accounted for the greater content of organisms as compared with the clasmatocyte. In this connection it is interesting to note that no fragmentation of the phagocytized organism was observed in the present study. Lurie[4] has recently indicated that monocytic cells from sensitized animals are more active in their ability to ingest carbon particles, tubercle bacilli and staphylococci than those cells obtained from normal animals. His experiment was performed *in vitro*.

These investigators arrived at their conclusions by observing microscopically the number and morphology of the organisms ingested by the cell under consideration. This leaves open the important question of the actual effect of phagocytosis upon the viability of *Mycobacterium tuberculosis* after having been engulfed *in vivo*.

Experimental. The phagocyte herein employed will be termed histiocytes (clasmatocytes, tissue macrophage, mononuclear cell). It seems inappropriate to use the term "monocyte" as the exact origin of that cell is at present unknown. In view of the fact that the so-called milky spots are the site of origin of many histiocytes, one would expect that the phagocytic cells found in the peritoneal fluid would be of a histiocytic nature.

The histiocytes were called out by injecting a sterile suspension of 500 mg of aleuronate in 15 cc of Ringer's solution into the peritoneal cavity of normal guinea pigs weighing about 500 g. After 72 hours a heavy homogeneous suspension of *Mycobacterium tuberculosis* was introduced into the same locality. Two strains were employed, namely the H37 and the more virulent H9096 which is also of human origin. All phagocytosis occurred *in vivo*. After 24 hours had elapsed the animal was killed by a sharp blow on the neck and the peritoneal cavity opened under sterile precautions. The fluid was washed out by introducing 15 cc of sterile heparin in Ringer's solution (0.1 g heparin in 200 cc of solution previously heated to 60 degrees for 15 minutes and filtered through a tested Berkefeld candle). Prior tests have indicated that heparin in far stronger concentrations has no effect on the viability of *Mycobacterium tuberculosis*. By employing the neutral red technic, vital stained preparations were made to determine the number of histiocytes present. Only one of the guinea pigs revealed less than 90%, the average being 93.9%. Films of the peritoneal fluid were then stained by the Ziehl-Neelsen method to ascertain the number of histiocytes which had engulfed the *Mycobacterium tuberculosis.* This range varied from 7% to 78%, the median being 32.5% and the average 34.8%. In one instance 78% of the cells had engulfed the organisms. Fifty percent of the peritoneal exudates revealed a phagocytosis rate of from 24% to 44%. Twenty-five percent of the fluids were found to have 45% to 78% of the cells engulfing organisms, while another 25% revealed a phagocytic index of from 7% to 23%.

---

[3] Borquist, M., and Rowe, C., *Am. Rev. Tuberc.*, 1931, **24**, 2.

[4] Lurie, M. B., *J. Exp. Med.*, 1939, **69**, 579.

By employing the single cell technic previously described[5, 6, 7] the histiocytes were isolated singly in microdroplets of Ringer's-heparin solution slightly tinted with neutral red. The cells were thoroughly washed by sucking up into a micropipette the fluid of the microdroplet surrounding the cell and by applying fresh fluid until no extracellular particles were visible under the oil immersion lens. As a further control, to make certain that the histiocyte had been washed free of unengulfed tubercle bacilli, the last wash fluids applied to the cells just prior to planting, were collected in a single micropipette from all of the microdroplets containing isolated histiocytes, and planted upon suitable medium. Finally, to determine that the technic and reagents employed did not affect the viability of the mycobacteria, a small droplet of the peritoneal fluid from each animal containing free organisms, was also planted.

The washed histiocytes were then collected in a fresh micropipette and planted upon slants of Corper's egg medium.

To determine whether the technic and method employed would support the growth of very few *Mycobacterium tuberculosis,* small numbers of organisms were isolated in microdroplets and counted under the oil immersion lens. The microdroplets were minute enough to be included within the circumference of the lens, and shallow, so that all parts could be focused upon. Moreover, the double micromanipulator was utilized so that one micropipette was in readiness to pick up the counted organisms, just as soon as the bacteria had been isolated by the other. Plants were made upon 111 slants of Corper's medium, and upon 98 of Petroff's. Thirty-two, or 28.8% of the slants of Corper's medium, on which from 2 to 33

---

[5] Kahn, M. C., *Am. Rev. Tuberc.*, 1929, **20**, 150.

[6] Kahn, M. C., and Schwarzkopf, H., *J. Bact.*, 1933, **25**, 157.

[7] Hotopp, M., and Kahn, M. C., *J. Infect. Dis.*, 1936, **58**, 324.

organisms were seeded, revealed growth, the average number of organisms for each tube was 13. From 2 to 28 organisms were seeded on 98 slants of Petroff's medium, the average number for each being 16.9. Eight of these tubes revealed growth or 8.2%. A similar series was made employing Petragnani medium, the results in our hands being somewhat better than those obtained on Petroff's. When from 25 to 100 tubercle bacilli were planted on Corper's medium, growth was obtained in 48% of the 52 tubes utilized.

This control did not favor the hypothetical bacteriostatic properties of the histiocyte as almost always more than one mycobacterium was observed in the cell, and clumps were frequently encountered. Also it is much easier to pick up a large body such as a histiocyte with the micropipette, and relatively few were lost in the micromanipulation, as must have been the case with the mycobacteria.

Experiments were performed 24 and 48 hours after the tubercle bacilli had been phagocytized. In other words, one plant was made upon the day upon which the guinea pig was sacrificed. The residual peritoneal fluid then being kept over night in the incubator at 37°C, and worked upon the day following.

Results. The following is a brief summary of this problem which has been under investigation for nearly 4 years. In the beginning of the experiment, washed histiocytes from guinea pigs were planted on Petroff's medium involving a total of 4398 cells, 1128 of which had phagocytized *Mycobacterium tuberculosis*. In view of the fact that the control experiments indicated Corper's egg medium to be so much more sensitive when small numbers of unengulfed bacteria were planted, the results obtained on Petroff's medium are considered invalid.

Employing Corper's egg medium, the peritoneal exudates of 32 guinea pigs were used and 4198 washed histiocytes were planted on 101 tubes of medium. The total number of histiocytes planted which had engulfed *Mycobacterium tuberculosis* was 1513 or 34.8% of those planted. The average number of histiocytes planted per tube of medium was 39.3.

It was found that the median percentage of cells engulfing tubercle bacilli was 32.5. The theoretical average number of histiocytes containing mycobacterium was 14 per tube of medium. Fifty percent of these tubes ranged from 9 to 19 microörganisms each, 25% ranged from 20 to 46, and 25% contained from 2 to 8 tubercle bacilli per tube.

Of the entire 101 tubes planted with histiocytes containing microorganisms only 2 slants or 2% of the total revealed growth; one of these tubes received a minimum of 36 ingested organisms while the other was seeded with a minimum of 20. Although these tubes received inoculations with numbers of bacteria that were above the average for the control (13 organisms), it must be taken into consideration that during the experiment one tube of medium received a minimum of 36 engulfed tubercle bacilli, 3 tubes received at least 40 organisms each and one was inoculated with at least 46 intracellular mycobacteria. None of these cultures gave rise to growth.

The mycobacteria contained within 3327 histiocytes were counted to determine the average number of organisms per cell. Twenty-eight percent of these histiocytes had engulfed clumps which were very conservatively estimated as 5 bacteria per clump. The number of *Mycobacterium tuberculosis* engulfed by the other 2311 histiocytes ranged from one to 10, the average being 3.5. Therefore, it will be observed that the results presented are of interest because in each case the conclusions are based upon counting only one organism per histiocyte, according to the phagocytic index.

After each day's work the wash waters left in all of the microdroplets which had contained histiocytes were collected in a micropipette and planted upon tubes of Corper's medium. This was done to test for the presence of unengulfed bacteria. Of the 101 tubes employed for such purpose, all remained sterile. Contrasted with this we find that of 32 tubes of media inoculated with a small droplet of unmanipulated peritoneal fluid, 31 revealed growth.

Summary. Under the experimental conditions described, it would seem that the histiocytes of normal guinea pigs are in many instances capable of exerting at least bacteriostatic properties toward engulfed *Mycobacterium tuberculosis* of the human variety when phagocytosis occurs *in vivo*. Of 101 tubes of Corper's egg medium seeded with a total of 4190 histiocytes, 1513 or 34.8% which had engulfed the mycobacteria, growth was obtained in only 2 instances, or 2%. When comparably small numbers of free *Mycobacterium tuberculosis* were inoculated upon the same medium, growth was obtained in 28.8% of the tubes employed. As no fragmentation of the bacteria within the histiocytes was observed, the bacteriostatic action exerted may be possibly explained as due to a disturbance in the metabolism of the engulfed *Mycobacterium tuberculosis*.

12088

Mobility Velocities of Histiocytes from Normal and from Sensitized Guinea Pigs.*†

Morton C. Kahn.

From the Department of Public Health and Preventive Medicine, Cornell University Medical College, New York City.

In a previous communication[1] it was shown that the electrophoretic mobility velocities of rough and of smooth avian *Mycobacterium tuberculosis* of the A-I and N strains varied considerably. The organisms from the smooth colonies were considerably more virulent and gave rise to a more rapid velocity across the electrical field. The results of the present communication indicate that the velocities of histiocytes (tissue phagocytes, macrophages, monocytic cells) obtained from guinea pigs sensitized with human *Mycobacterium tuberculosis* of the H37 strain and those from normal animals also differ, the sensitized animals revealing the reverse of that obtained with the smooth type of avian organisms, namely a slower rate on the whole.

The histiocytes were obtained by injecting each animal intraperitoneally with a sterile suspension of aleuronat (500 mg in 15 cc of Ringer's solution) and about 72 hours later one-half of this dose was injected in the same region. Eighteen hours after the second injection the animal was sacrificed by a blow on the back of the neck and the peritoneal cavity opened under sterile precautions. Twenty-five cc of a 25% solution of sterile sucrose solution was then introduced into the cavity by employing a syringe with a piece of glass tubing attached by means of rubber tubing. The animal was gently tilted from side to side to insure thorough mixing of the fluids. Sucrose solution was employed in this experiment because it is non-electrolytic, prevented clotting, and, moreover, hardened the cells so that they did not degenerate under subsequent manipulation. A series of experiments indicated that sucrose in the concentration above mentioned was optimal for the purpose at hand. The exudate and sucrose solution mixture was then centrifuged in a Bolaget Winkle angle centrifuge for some 20 minutes at a speed of about 3000 r.p.m., which was sufficient to deposit the vast majority of the cells. This process was repeated 3 times, adding fresh 25% sucrose solution and thoroughly mixing the cells after each centrifuging. In order to remove as much electrolyte as possible the cells were then washed 4 times by centrifuging employing sterile distilled water.

---

* This study is part of a group investigation being carried on in coöperation with the Medical Research Committee of the National Tuberculosis Association.

† With the technical assistance of Ann Sonnentheil.

[1] Kahn, M. C., and Schwarzkopf, H., *Am. Rev. Tuberc.*, 1931, **23**, 45.

Prior to these manipulations, vital neutral red preparations were made in order to determine the percentage of histiocytes present in each exudate. The counts varied from 85 to 100%. The exudates of only 3 of the guinea pigs employed gave a count below 95%.

The apparatus and technic for determining the mobility velocities has been previously described.[1, 2] A high dry lens was employed (8 mm 0.50, × 21). All readings were gathered from observations made in the lower level of the apparatus, as this zone was found to be the more constant. The apparatus was filled with 5 separate samples from each peritoneal exudate and only 12 readings were taken from each one. The lower zone level could not be calculated individually for each set-up as the time necessary for this procedure would have permitted many of the histiocytes to sink to the bottom of the glass chamber; therefore, the number of revolutions of the fine adjustment of the microscope was computed several times for each of the 5 glass chambers employed and an average thus arrived at. There was only a negligible variation and constant results were obtained in calibration.

Ten guinea pigs were injected with 0.01 mg of living *Mycobacterium tuberculosis* of the H37 strain. The suspension of organisms was made according to Corper's method by sterilely lifting the growth membrane from Long's liquid medium on to pieces of sterile filter paper, thus allowing the filter paper to absorb as much of the liquid as possible. This partially dried membrane was transferred to a sterile mortar and triturated with about 15 drops of 0.5% sodium taurocholate solution until the mass was reduced to a homogeneous pasty consistence. Ten cc of sterile distilled water was then gradually added and grinding continued further. The suspension was allowed to settle in the mortar and the supernatant fluid pipetted into a test tube and the organisms were allowed to sediment further. The top portion of this fluid containing mycobacteria was transferred to another test tube and exactly one cc of this was pipetted to a sterile weighed watch crystal, evaporated to dryness over a hot water bath and weighed. The suspension was then diluted with sterile physiological saline solution until each cc contained 0.01 mg of organisms. Injections into the guinea pigs were made subcutaneously and sen-

---

[2] Falk, I. S., and Jensen, L. B., *J. Bact.*, 1928, **15**, 367.

sitization was proven by applying the tuberculin test with purified protein derivative. Nine animals reacted positively and from these a total of 500 histiocytes were tested.

Histiocytes in the sensitized normal animals were called out in the same fashion as already described for the normal animals, and the count as made by the vital neutral red method revealed about the same percentage of cells for each animal. The exudates from 20 normal guinea pigs were employed involving a total of 954 histiocytes.

For both experiments the source of current was two 45-volt Standard Layer-bilt Radio "B" batteries connected in series. This produced 90 volts as elicited by the voltmeter prior to each experiment.

TABLE I.

Velocity Statistics of Histiocytes from Normal and from Sensitized Guinea Pigs.

	Normal sec	Sensitized sec
Range	2.0 to 7.0	2.4 to 6.6
Mean	3.82	4.32
Median	3.80	4.20
Lower Quartile	4.4 to 7.0	5.0 to 6.6
Upper "	2.0 to 3.2	2.4 to 3.6
Avg Dev. from Mean	0.758	0.788
Possible Range of Mean	3.77 to 3.87	4.25 to 4.39
Standard Error	0.024	0.035

Results. The velocities of the histiocytes obtained from both the normal and sensitized animals were measured across an electrical field which measured 360 micra. As far as the 954 cells from the normal guinea pigs are concerned, the velocities ranged from 2.0 to 7.0 seconds. The mean of the speed was found to be 3.82 seconds and the median 3.8 seconds. The lower quartile ranged from 4.4 to 4.0 seconds, while the upper quartile range was from 2.0 to 3.2 seconds. The average deviation from the mean was found to be 0.7581 seconds. The standard error was calculated as 0.0245. The possible range of the mean velocities was 3.77 to 3.87 seconds.

In comparison it is interesting to note the data gathered for the 499 histiocytes from those animals sensitized with *Mycobacterium tuberculosis.* These velocities ranged from 2.4 to 6.6 seconds. The mean was found to be 4.32 seconds and the median, 4.2 seconds. The range of the lower quartile was 5.0 to 6.6 seconds and that of the upper quartile 2.4 to 3.6 seconds. The average deviation from the mean was 0.7884 seconds and the standard error 0.0353. The possible range of the mean velocities of these histiocytes obtained from the sensitized guinea pigs was found to be 4.25 to 4.39 seconds.

Summary. With the technic described the mobility velocities of histiocytes obtained from normal guinea pigs were compared with those obtained from guinea pigs sensitized with the H37 strain of living *Mycobacterium tuberculosis.* The cells of the infected animals revealed a trend toward a slower rate. Statistically the difference obtained is significant as the range of the mean of the two in no point coincides. These findings may indicate a difference in surface charge.

12089 P

Growth of Louse-Borne Relapsing Fever Spirochetes in Chick Embryo.

Kuo-ching Chen. (Introduced by Samuel H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

It is well known that important biological differences exist between the tick-borne and louse-borne relapsing fever spirochetes. With the former, infection can readily be produced in a large variety of laboratory animals which together with the tick, serve as means of keeping the strain in the laboratory. On the other hand, few animals were found to be susceptible to the louse-borne type, and the lice cannot be used to maintain the spirochetes. Although Meleney[1] was able to produce infection and relapses in squirrels and chipmunks with a Chinese louse-borne strain of relapsing fever spirochetes, splenectomy of these animals has to be resorted to in order to assure complete success. It is, therefore, of more than academic interest to determine if the louse-borne strains can also be grown and passed in the embryonic chick as is possible for the tick-borne spirochetes,[2, 3, 4] and the preliminary results are herewith communicated.

Blood samples were obtained from 7 patients with louse-borne relapsing fever[5, 6, 7] during the febrile stage. Blood was either defibrinated or mixed with sodium citrate, and some samples have been kept in the refrigerator for 2 days before inoculation. Inoculation is made at the pointed end of the egg where a hole in the outer shell was made by a dental drill and sterilized with alcohol followed by flaming. The needle was inserted horizontally either deep into the yolk sac or about 1 cm beneath the chorio-allantoid membrane. About 0.2 cc of infective blood was used for the inoculum. Hard paraffin was used for the sealing. A series of fertilized eggs of from 5-12 days old was inoculated with each sample of blood and examinations were made daily from the second day onward for spirochetes in blood, amniotic fluid, yolk and emulsions of internal organs.

---

[1] Meleney, H. E., *J. Exp. Med.*, 1928, **48**, 65.

[2] Oag, R. K., *J. Path. and Bact.*, 1939, **49**, 339.

[3] Chabaud, A., *Bull. Soc. Path. Exot.*, 1939, **32**, 483.

[4] Bohls, S. W., Irons, J. V., and Shazo, T. D., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 375.

Results. Good growth of spirochetes was seen in eggs from the third day for all the 7 blood samples inoculated, but the maximum growth was most commonly found on the fifth day of infection. The number of organisms was, however, greatly increased after being passed for 3 generations by means of the blood of embryonic chick, but the time of their appearance was not shortened. In agreement with previous workers, infection was limited almost entirely to the blood. Furthermore, soon after the death of the embryo, all the spirochetes were found to have lost their motility, and eventually disappeared. It is evident, therefore, that the multiplication and maintenance of the louse-borne spirochetes in the developing egg depend strictly upon the living embryo. In this sense, the embryonic chick may perhaps be considered and employed as a susceptible animal for the growth and passage of louse-borne relapsing fever spirochetes.

---

[5] Robertson, R. C., *Chinese Med. J.*, 1932, **46**, 853.

[6] Chung, H. L., *Chinese Med. J.*, 1936, **50**, 1723.

[7] Chung, H. L., and Wei, Y. L., *Am. J. Trop. Med.*, 1938, **18**, 661.

12090 P

Fragility of Erythrocytes as Affected by Anoxia, CO_2 Inhalation and Insulin Hypoglycemia.

MARGUERITE BOOTH. (Introduced by Irvine McQuarrie.)
From the Department of Pediatrics, University of Minnesota, Minneapolis.

In the course of studies on the mechanism of insulin convulsions, McQuarrie and Ziegler[1] frequently observed marked hemolysis in blood samples drawn during the later stages of experiments involving severe anoxia or hypoglycemia. The present investigation was undertaken in an attempt to determine the nature of the hemolytic mechanism responsible for this effect. The current tendency to widespread use of insulin shock and anoxia in the treatment of schizophrenia and certain other neuropsychiatric disorders lends added significance to the problem. While other hematological changes were also investigated, alterations in the fragility of the erythrocytes alone are considered here.

Most of the experiments were carried out on full-grown normal dogs. A number of these were repeated, however, after bilateral adrenalectomy had been performed on the animals to render them more sensitive to insulin. The adrenalectomized animals were kept in comparatively good condition by daily injections of cortin and by use of a diet of low potassium and high sodium content. All dogs were fasted for at least 18 hours before experiments were begun. Erythrocyte fragility tests were made on freshly drawn venous blood by the method of Todd and Sanford[2] immediately before and at intervals of 30 to 60 minutes during prolonged periods of exposure to atmospheres varying widely in their partial pressures of O_2, N_2, and CO_2. When gaseous mixtures, other than room air, were used, they were mixed and adjusted at atmospheric pressure in an ordinary oxygen tent connected with separate tanks of N_2, O_2, and CO_2. As an added check on the partial pressures of the gases in the tent, rough analyses were made at 30-minute intervals throughout each experiment.

Thirty-one experiments were carried out on 9 dogs as follows: Twelve with a gaseous mixture of 5% O_2 and 95% N_2 (3 after adrenalectomy); 2 (lasting but 2 minutes) with 1% O_2 and 99%

---

[1] McQuarrie, I., and Ziegler, M. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 525.

[2] Todd, J. C., and Sanford, A. H., *Clinical Diagnosis by Laboratory Methods*, 8th ed., revised, Saunders, Philadelphia, 1937.

N_2; 5 with 15% CO_2, 20% O_2, and 65% N_2 (3 in normal and 2 in adrenalectomized animals); 3 with 90% O_2 and 10% N_2; 1 with 15% CO_2, 70% O_2, and 15% N_2; 7 involving marked insulin hypoglycemia (in room air) and 1 involving both anoxia (5% O_2) and insulin hypoglycemia. These experiments were controlled by blood sampling at similar intervals in room air. To ascertain the range of erythrocyte fragility for normal dogs breathing room air, 48 determinations were carried out in 19 different animals. The initial hemolysis in the latter series of dogs varied from 0.40% to 0.58% of NaCl solution, and complete hemolysis from 0.26% to 0.42%. None of the dogs used in the experiments showed hemolysis at these higher concentrations while breathing room air.

Breathing atmospheres containing high percentages of O_2 had no measurable influence on the fragility of the erythrocytes. Adrenalectomy *per se* likewise had no effect. However, breathing an atmosphere containing but 5% O_2 over a period of several hours produced a marked increase in fragility in all animals so exposed. Initial hemolysis occurred at concentrations of NaCl solution as high as 0.70% in some animals and well above 0.60% in the majority. An atmosphere containing 15% CO_2 increased the fragility of the red blood cells by 0.04 to 0.10% in normal and also in adrenalectomized dogs. Prolonged, severe insulin hypoglycemia increased the fragility of the erythrocytes by 0.04 to 0.08% saline solution. This effect was accentuated by simultaneous exposure of the animal to an atmosphere containing but 5% O_2

Conclusion. From the foregoing results it is concluded that the erythrocytes are severely injured, as evidenced by their increased fragility, by prolonged anoxia, by exposure to a high CO_2 atmosphere and to a less degree by long periods of insulin hypoglycemia.

12091

Reaction of Folin's Reagent with Proteins and Biuret Compounds in Presence of Cupric Ion.

ROGER M. HERRIOTT. (Introduced by J. H. Northrop.)

From the Laboratories of the Rockefeller Institute for Medical Research, Princeton, N.J.

In an earlier paper[1] the writer noted that traces of copper sulfate increased the blue color obtained by treating such proteins as pepsin or gelatin with Folin's phenol reagent.

It has now been found that a number of organic substances reduce the Folin's phenol reagent only if a small amount of cupric ion is present. These same substances also show a positive biuret reaction.

The copper-phenol values in Table I represent the amount of color per milligram of substance produced as a result of adding M/1300 copper sulfate to the solution under examination with the phenol reagent. One obtains this value from the difference in color values produced with and without copper ion. For many substances no color is produced in the absence of copper but most proteins contain tyrosine and tryptophane and must, therefore, be analyzed with and without cupric ion. No attempt has been made to determine whether each of the substances in Table I produces a blue color exactly in proportion to its concentration. With leucyl-glycyl-glycine the proportionality is quite good while with pure biuret it is not as good. The order of magnitude of the values in Table I is of more significance than the absolute values. The solutions have been read against a tyrosine standard and the values calculated as the number of milligrams of tyrosine that will give the same amount of color under identical conditions.

No amino acids show any comparable effect of the presence of cupric ion when tested with the phenol reagent.

With certain substances, particularly biuret, the presence of some non-chromogenic substances has fairly pronounced effect on the amount of blue color that develops in the presence of cupric ion. Thus the presence of glycine or glycyl-glycine increases the copper-phenol color of biuret. Urea gives a blue color with the phenol reagent in the presence of copper ion and will, therefore, interfere if present in a solution being analyzed.

---

[1] Herriott, R. M., *J. Gen. Physiol.*, 1935, **19**, 287.

TABLE I.

Substance	Amount used, mg	Copper-phenol color value per mg material,* mg tyrosine
Tyrosine	0.15	0
Biuret†	0.1	1.3
Casein (Hammarsten)	0.84	.13
Zein	0.81	.17
Egg albumin	1.05	.15
Edestin	0.74	.23
Gliadin "B"	0.63	.22
Pepsin	0.53	.20
Pepsin (autolysate)	0.53	.24
Pepsin (acid hydrolysate)	1.0	.02
Gelatin	1.0	.14
Gelatin (acid hydrolysate)	10.5	.02
Peptone (Roche)	0.5	.12
Glycyl-tyrosine	0.18	.0
Glycyl-tryptophane	0.4	.0
Glycyl-leucine†	18.9	.005
Glycyl-glycine†	13.2	.015
Malonamide†	1.0	.25
Oxamide†	0.9	.20
Formamide†	4.5	.0
Leucyl-glycyl-glycine†	0.25	.2
Histidine	4.6	.02

*This value is obtained as the difference between the value in the presence of copper ion and in the absence of it, divided by the milligrams of material used.

†These substances show practically no color in the absence of copper ion.

In view of the fact that the products of hydrolysis have little if any influence on the color of leucyl-glycyl-glycine, it seems likely that one could follow quantitatively the extent of enzymatic hydrolysis of this and similar peptides by means of this color test.

The amount of copper required for maximal color development varies somewhat with different substances but it has been found that 1 ml of M/100 copper sulphate is maximal or nearly so for most of the different substances thus far used.

Very little can be said about the mechanism of the reaction but it should be recalled that Wu[2] pointed out many years ago that as little as one part in five million of cuprous copper ion reduces the phenol reagent.

These experiments demonstrate that under certain conditions proteins or peptides which seem unreactive may, in the presence of very small amounts of a second substance, exhibit very marked reactivity.

Experimental Details. Procedure. One ml of a solution of the substance to be studied was put into a 50 ml Erlenmeyer flask along with 1 ml of M/100 copper sulphate and 8 ml of 0.5 N sodium hydroxide. To this was then added dropwise with whirling 3 ml of the ⅓ dilution of Folin's[3] phenol reagent. The solutions were then read after 5-10 minutes against 0.15 mg tyrosine treated in a similar manner.

---

[2] Wu, H., *J. Biol. Chem.*, 1920, **43**, 189.

12092

Use of Anterior Lobe of Prostate Gland in the Assay of Metakentrin.

R. O. GREEP, H. B. VAN DYKE AND BACON F. CHOW.

From the Division of Pharmacology, the Squibb Institute for Medical Research, New Brunswick, N.J.

We believe that the recent isolation of a homogeneous protein, from pituitary tissue, which has the property of causing the formation of corpora lutea from preformed graafian follicles and of stimulating ovarian and testicular interstitial cells in rats establishes the individuality of a gonadotrophin with some but not all the properties of luteinizing hormone as originally recognized by Fevold, Hisaw and their coworkers. During the course of our purification procedures, we have utilized the weight increase of the anterior lobe of the prostate gland in the assay of the interstitial cell-stimulating hormone[1, 2, 3]—metakentrin.[4] The principal objection to this procedure lay in the possibility that the substance stimulating the secretion of androgen by the interstitial cells of the male gonad was not identical with the substance causing formation of corpora lutea. Following the isolation of metakentrin[1] we have determined that under proper conditions all these biological effects are manifestations of this pure preparation. We are prompted, therefore, to record our experience and technic in assaying this hormone by the ventral prostate method.

Our standard procedure is as follows: The animals used are hypophysectomized immature male rats. The operation is performed at 21-22 days of age, body weight being selected to fall between 35 and 47 g. Injections are started after a 2-day postoperative interval and are continued for 4 days with autopsy on the 5th day. One cubic centimeter of solution representing one-fourth of the total dose is administered subcutaneously once daily for 4 days. Removal of the anterior prostate free from surrounding connective tissue and fat can be easily accomplished; dissection of definitely atrophic glands is facilitated by the use of a dissecting binocular. The glands are placed in saline from which they are removed and weighed on a torsion balance (accuracy ±0.02 mg) after excess of fluid has been quickly absorbed by filter paper.

---

[3] Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627.

[1] Shedlovsky, T., Rothen, A., Greep, R. O., van Dyke, H. B., and Chow, B. F., *Science,* 1940, **92**, 178.

[2] Chow, B. F., Greep, R. O., and van Dyke, H. B., *J. Endocrinol.*, 1939, **1**, 439.

[3] Greep, R. O., Chow, B. F., and van Dyke, H. B., *J. Biol. Chem.*, 1940, **133**, 289.

[4] Coffin, H. C., and van Dyke, H. B., *Science,* 1941, **93**, 61.

Control organs were taken from untreated animals killed 6 days after hypophysectomy. The weights of the anterior prostate and the testes of such animals fall within close limits. For example, the mean and standard error of the weight of the anterior prostate of 57 control rats were 6.16 ± 0.118 mg. Similar data with respect to the pairs of testes of these animals were 109.6 ± 1.11 mg. Our experience indicates that only occasionally rather than routinely is it necessary to include a control group with other groups receiving hormone. A minimum of 5 animals per dose is satisfactory for routine tests but for critical assay, at least 10-20 animals per dose is desirable.

Fevold[5] uses the hypertrophy of the seminal vesicles of normal immature male rats as a means of assaying luteinizing hormone. Chow, Greep, and van Dyke[2] were unable to produce gross stimulation of the seminal vesicles in hypophysectomized immature males after the injection of amounts of hormone causing great enlargement of the anterior prostate. The data in Table I show the marked response of the prostate as well as the virtual lack of response of the seminal vesicles of the hypophysectomized immature male rat to testicular secretion induced by a pituitary extract in which the gonadotrophins had not been fractionated. Many experiments were also performed in which the period of injection was 8 days instead of 4; these are recorded in Table I. A dose (75.0 mg equivalent of fresh gland) 6 times greater than was required to produce a significant enlargement of the anterior prostate failed to enlarge the seminal vesicles. Moreover, there was no correlation between dose of extract and response of seminal vesicles, whereas good correlation was found with respect to the anterior prostate. (Work reported from the laboratory of H. M. Evans likewise failed to demonstrate that changes in the seminal vesicles can be

---

[5] Fevold, H. L., *Cold Spring Harbor Symposia*, 1937, **5**, 93.

TABLE I.
Effect of Unfractionated Gonad-stimulating Extract of Hog Pituitary on Testes and Accessory Organs of Hypophysectomized Immature Rats.

	Treatment			Mean and S. E. of wt of		
No. rats	Days	Total mg eq. fr. gl.	Avg body wt, g	Testes, mg	Ant. prost., mg	Seminal vesicles,* mg
17	—	—	38.4	102.4± 2.96	7.83±0.299	6.24±0.200
17	4	12.5	34.3	135.2± 6.19	12.75±0.709	6.52±0.173
17	4	25.0	40.1	191.5± 6.37	15.09±0.912	7.24±0.196
17	4	75.0	38.3	219.5± 9.51	18.08±0.770	7.29±0.256
17	4	150.0	38.3	245.4± 7.58	20.48±1.201	7.69±0.257
17	—	—	40.3	81.1± 4.34	5.44±0.191	6.00±0.238
15	8	12.5	43.0	125.7± 6.23	12.97±1.369	7.16±0.241
16	8	25.0	42.1	132.7± 3.72	11.92±0.651	6.05±0.209
15	8	25.0†	42.0	172.8± 8.88	15.29±1.077	6.81±0.177
15	8	37.5	43.6	209.0±13.52	17.45±1.384	7.10±0.851
16	8	50.0	41.6	212.5± 7.70	15.87±0.898	6.01±0.152
16	8	75.0	42.5	252.4±10.71	20.54±1.409	6.37±0.347
17	8	150.0	42.7	321.7± 9.92	22.86±1.165	7.42±0.211
18	8	300.0	42.7	328.6±14.43	25.99±1.906	8.35±0.429
15	8	600.0	40.8	338.6± 9.44	28.48±2.242	7.78±0.352
17	8	1000.0	44.5	390.0±14.05	38.25±2.998	9.11±0.526
16	8	3500.0	43.8	371.5±10.77	41.87±2.650	7.63±0.186

*Including coagulatory glands.
†Intraperitoneally; all others subcutaneously.

satisfactorily used for the assay of interstitial cell-stimulating hormone.) Our attempts to produce a response in the seminal vesicles of normal immature males by this means have also met with poor success (Table II). The anterior prostate of normal rats undergoes hypertrophy but we cannot recommend its use in the assay of metakentrin; the amount of prostate growth seen in untreated control animals shows how seriously the presence of the test animal's own pituitary may complicate the assay.

Fevold[6] has concluded that the stimulating effect of luteinizing

TABLE II.
Effect of Unfractionated Gonad-stimulating Extract of Hog Pituitary on Testes and Accessory Organs of Normal Immature Rats. Injections Were Made Intraperitoneally.

	Treatment			Necropsy: Mean wt of			
No. rats	Days	Total g eq. fr. gl.	Age, days	Body, g	Testes, mg	Ant. prost., mg	Sem. Ves.,* mg
11	—	—	21	38.5	175.1	24.10	8.30
11	—	—	25	45.8	309.0	29.00	8.00
8	4	5	25	44.4	405.1	47.85	10.12

*Including coagulatory glands.

---

[6] Fevold, H. L., *J. Biol. Chem.*, 1939, **128**, 83.

hormone, derived from sheep pituitaries, on the seminal vesicles of normal or hypophysectomized immature males is potentiated by the administration of follicle-stimulating hormone of similar origin. It is clearly possible to interpret this observation in several ways. We wish only to emphasize that the assay technic here recommended is specific for metakentrin and is not influenced by the simultaneous administration of biologically pure thylakentrin* (Table III).

TABLE III.
Lack of Effect of Thylakentrin (FSH) on Response of Prostate of Hypophysectomized Immature Rats Receiving Metakentrin (ICSH).
(Hormones were combined *in vitro*.)

No. rats	Dose in μg N		Mean wt	
	Metakentrin	Thylakentrin	Testes, mg	Ant. prost., mg
4	0	0	116.1	6.86
4	0	20	187.9	7.89
6	4	0	128.4	10.02
5	4	20	187.4	11.36
4	0	30	204.4	7.25
5	2	0	148.7	11.48
6	2.5	30	197.7	10.41
4	5	0	124.7	13.77
5	5	30	212.8	11.87
8	2	0	130.8	10.77
7	2	50	222.9	11.94

Evans, Korpi, Simpson, Pencharz and Wonder[7] observed that the atrophic interstitial cells of hypophysectomized female rats were repaired by their ICSH fraction but not by other pituitary hormones. Their earlier view that the interstitial cell-stimulating and luteinizing hormones are not identical has been withdrawn[8, 9] and the repair of the interstitial cells of hypophysectomized female rats is now used by them in the assay of luteinizing hormone. The relative sensitivity of this technic as compared with ours has not been accurately determined. Our data, however, indicate that the ovarian interstitial cells, judged by alterations in their histology, are somewhat less sensitive toward metakentrin stimulation than are the interstitial cells of the testes judged by the secondary effect of secreted androgen on the anterior prostate.

---

* This name was introduced by Coffin and van Dyke[4] to refer to pure-follicle-stimulating hormone whose action appears to be solely to stimulate follicle growth unaccompanied by estrogen secretion unless metakentrin also is administered.

[7] Evans, H. M., Korpi, K., Simpson, M. E., Pencharz, R. I., and Wonder, D. H., *Univ. Calif. Pub. in Anat.*, 1936, **1**, 255.

[8] Jensen, H., Simpson, M. E., Tolksdorf, S., and Evans, H. M., *Endocrinology*, 1939, **25**, 57.

[9] Evans, H. M., Simpson, M. E., Tolksdorf, S., and Jensen, H., *Endocrinology*, 1939, **25**, 529.

Fig. 1 summarizes graphically our experience with various doses of chemically pure metakentrin assayed by the method described in this report. Each point represents an average of 7.4 animals. Corresponding points such as open circles, crosses, etc., represent assays on one preparation carried out simultaneously at 2-3 dose-levels. The formula for the straight line was calculated by the method of least squares.

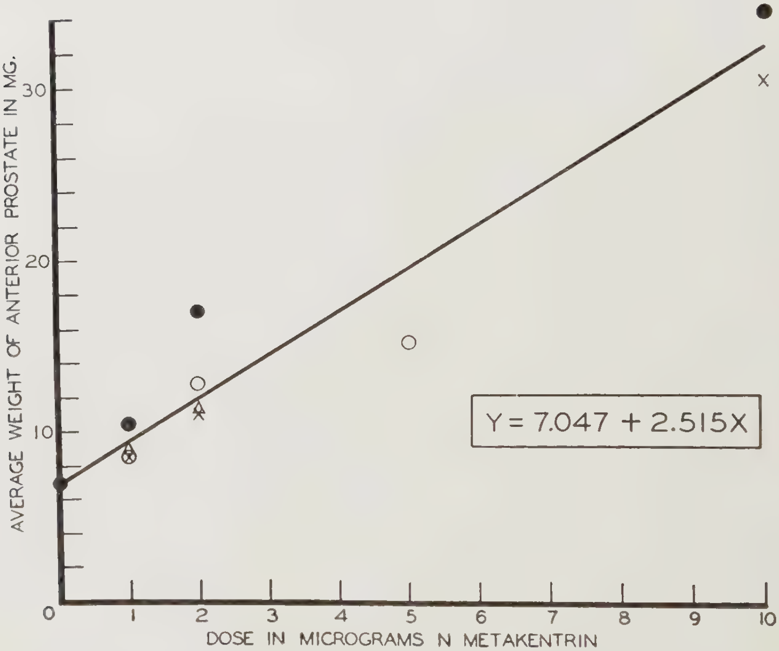


FIG. 1.

Dose-response curve of anterior prostate of hypophysectomized immature rats receiving total doses of 1-10 μg of N of pure metakentrin of which one-fourth was administered daily for 4 days. See also text.

The chief advantages in using the anterior prostate in the assay of metakentrin are: 1. The assay can be read objectively. 2. The assay can be placed on a quantitative basis. 3. No histological preparations or evaluations are required. 4. The presence of thylakentrin does not influence the assay. The chief disadvantages are: 1. The assay is not qualitative in that it does not show whether or not the preparation is free of thylakentrin. This must be determined by the use of hypophysectomized female rats unless the extract is free from metakentrin; in such a case, male rats can be used and thylakentrin causes only testicular hypertrophy. 2. Prostatic enlargement being an indirect response mediated through the testes is subject to greater variability than would be expected were the effect direct as when prostate stimulation is produced by injected androgen.

Summary. In the assay of pituitary gonadotrophins, the enlargement of the anterior prostate of hypophysectomized immature male rats not only may be used as a specific test for metakentrin (ICSH), but also may be employed satisfactorily in making a quantitative assay of this substance.

12093 P

Deposition of Melanin in Sparrow Bill Following Local Action of Testosterone Propionate in Alcoholic Solution.*

Arthur Kirschbaum and Carroll A. Pfeiffer. (Introduced by Edgar Allen.)

From the Department of Anatomy and the Adolescence Study Unit, Yale University School of Medicine, New Haven, Conn.

The bill of the English sparrow is very sensitive to the action of androgens.[1, 2, 3] Intramuscular injections of androgens dissolved in oil have been shown to induce pigmentation of the bill in the castrate male, or in sexually inactive birds of either sex.[1, 2, 3] During the breeding season the bill of the male is jet black. As the testis enlarges (during the winter months) previous to mating activity the bill changes progressively from the yellow-color of the juvenal or the sexually inactive adult to the deep black of the sexually active male.[2, 3, 4] The bill of the female also becomes appreciably darker during the egg-laying season; this response is due to secretion of ovarian androgen and not to estrogenic hormone of the ovary.[5]

The demonstration of the local action of sex hormones indicates

---

* Aided by grants from the Rockefeller Foundation, the Fluid Research Fund of Yale University School of Medicine, and the Committee for Research in Problems of Sex, of the National Research Council, the latter grant administered by Professor Edgar Allen.

[1] Keck, W. N., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1140.

[2] Keck, W. N., *J. Exp. Zool.*, 1934, **67**, 315.

[3] Witschi, E., Proc. Soc. Exp. Biol. and Med., 1936, **33**, 484.

[4] Kirschbaum, A., and Ringeon, A. R., *Anat. Rec.*, 1936, **64**, 453.

[5] Pfeiffer, C. A., and Kirschbaum, A., *Yale J. Biol. and Med.*, 1941, **13**, 315.

that they may in some cases act directly on effector cells rather than systemically or through some remote influence, for example, by way of the hypophysis. Such local reactions are growth phenomena; *e.g.*, growth of the mammary gland,[6-9] growth of the chick's comb,[10, 11] and vaginal epithelial growth.[12] To determine whether a phenomenon such as deposition of melanin (in the case of the sparrow bill under the control of androgenic hormone) can be influenced by direct (local) action of the hormone on the cells capable of producing the pigment, testosterone propionate[†] dissolved in absolute alcohol was applied locally to the bill-producing cells. These are situated at the angle of the bill.

A total of 17 birds (sexually inactive females with yellow bills) were used in these experiments. The hormone solutions were prepared so that a daily dose was contained in one drop of alcohol delivered from a 22-gauge hypodermic needle. In those instances in which the hormone was to act systemically, the solution was applied to the skin of the breast from which the feathers had been plucked. In determining local reaction the lowest dose (2 μg daily) was applied to the skin at the angle of the bill on the right side, the left side serving as a control.

Table I summarizes the results.

Whenever response was elicited following the application of hormone to the skin of the breast a diffuse deposit of melanin appeared throughout the bill, with greatest concentration at the angle of the bill and along the buccal margin. After local applications for 30 days the pigmented area was confined exclusively to the bill angle where the hormone was applied. In the latter case the pigment appeared first as a narrow black line of maximum intensity; as the pigmented area increased in size following further treatment it was sharply demarcated from the non-pigmented portion of the bill. This is further indication of the strictly local response.

---

[6] MacBryde, C. M., *J. A. M. A.*, 1937, **112**, 1043.

[7] Lyons, W. R., and Sako, Y., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 398.

[8] Speert, H., *Science*, 1940, **92**, 461.

[9] Gardner, W. U., and Chamberlain, T. L., in press.

[10] Fussgänger, R., Medicine in Its Chemical Aspect; Reports from the Medico-chemical Research Laboratories of the I. G. Farbenindustrie aktiengesellschaft 1934, **2**, 185, I. G. Farbenindustrie, AG Leverkusen, Germany.

[11] Frank, R. T., and Klempner, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 763.

[12] Stadler, L. B., and Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 562.

[†] The testosterone propionate was kindly furnished by Schering Corporation through the courtesy of Dr. E. Schwenk.

TABLE I.

No. of birds	Treatment	Dose of hormone	Latent period preceding bill pigmentation, days	No. with pigmentation response
4	systemic	8 μg	5-13	3
5	,,	4 μg	17-19	3*
3	,,	2 μg	25	1†
5	local	2 μg	4-5	5‡

*Very slight response in 1 animal.
† ,, ,, ,,
‡Heavy pigmentation in area at base of bill. No pigmentation on opposite (untreated) side.

Conclusion. Androgenic hormone can act locally to induce deposition of melanin in those cells of the sparrow bill which are capable of producing this pigment.

12094 P

Results of Daily Intravenous Injections of Lanatoside C Upon the Heart Muscles of Dogs.

JOHN S. LA DUE. (Introduced by George Fahr.)
From the Department of Internal Medicine, University of Minnesota.

Bauer, Hu, Buchner and Lindner[1] demonstrated that the daily intravenous administration of one-third or more of a cat unit* of digitoxin per kilo of body weight produces degenerative changes in the heart muscles of dogs in from 5 to 30 days. The coronary arteries of the hearts of the dogs studied were free of any gross or microscopic pathology, but small infarctions and areas of connective tissue replacement were found in the myocardiums.

In repeating these experiments, we gave lanatoside C,† a crystalline derivative of digitalis lanata, by vein to dogs and also attempted to determine the effects, if any, of moderate and large oral doses both of lanatoside C and of digitalis purpurea.

In July, 1938, 9 dogs were digitalized by vein according to their weight. Thereafter, doses of from one-half to 5 cat units of both preparations were administered once daily with the food. Each tablet of lanatoside C contained 0.25 mg and each cc of solution 0.20 mg; 0.281 mg of lanatoside C are equivalent to one Hatcher-Brody cat unit. Each 1½ grain tablet of digitalis purpurea contained one Hatcher-Brody cat unit.

---

[1] Bauer, F., *Arch. exp. Path. Pharm.*, 1934, **176**, 74; Hu, C., Lien, V., and Li, R., *Chinese Med. J.*, Suppl., 1936, **1**, 31; Büchner, F., *Arch. exp. Path. Pharm.*, 1934, **176**, 59; Lindner, W., *Arch. exp. Path. Pharm.*, 1938, **190**, 210.

* "Cat unit" always refers to the Hatcher-Brody unit.

†Cedilanid, Sandoz Chemical Works, Inc.

After 30 to 370 days, the animals were sacrificed and representative sections of the myocardiums secured. There were no abnormal gross findings and the microscopic sections were within normal limits.

Twenty-three dogs were given daily intravenous injections of lanatoside C ranging from one-twentieth to three-quarters of a cat unit per kilo of body weight. Nine of these animals died 6 to 12 hours before autopsy and post-mortem changes were too extensive to permit accurate conclusions.

All the animals receiving more than 0.20 of a cat unit per kilo of weight exhibited severe symptoms of intoxication. They developed anorexia, emesis, weight loss, salivation, muscular weakness and attacks of severe dyspnea. The dyspnea was noted late in the experiments, occurred 5 to 10 minutes after the injection of lanatoside C and persisted for from 5 to 45 minutes. It was usually associated with tachycardia or bradycardia and in one instance with a venous pressure of 15 cm of sodium citrate.

The coronary arteries of the hearts of all the dogs were free of any gross or microscopic changes. Subendocardial hemorrhages

TABLE I.
Experiments with Daily Intravenous Injections of Lanatoside C Correlated with Electrocardiography.

Dog No.	Daily dosage in cc	Dosage in cat units per kilo	Duration of experiment, days	Symptoms noted during experiments	EKG changes	Heart muscle pathology
1E	5.0	.25	8	anorexia, emesis wt loss, salivation, dyspnea, muscular weakness	present	none
2E	6.9	.33	8	same as 1E	”	”
3E	12.7	.50	4 (D)	” ” ”	”	?
4E	12.6	.75	4 (D)	” ” ”	”	?
5E	3.0	.20	60	same as 1E, except for absence of dyspnea	”	present
6E	6.0	.25	22	same as 5E	”	”
7E	8.0	.33	7	” ” 1E	”	”
8E	13.6	.50	7 (D)	” ” ”	”	?
9E	9.6	.40	8	” ” ”	”	none

(D) Represents animals dying 6-12 hours before autopsy.
Each cc of solution contains 0.20 mg of crystalline lanatoside C.

were seen in one of the hearts which was otherwise microscopically normal and in 3 of the hearts showing widespread cellular damage. There were no other significant gross autopsy findings in any of the dogs. (Table I.)

The hearts of 4 dogs receiving from one-fifth to one-half a cat unit per kilo of body weight over a period of from 16 to 33 days exhibited diffuse and widespread cellular damage. Numerous small infarctions containing a few mononuclear cells and very occasional polymorphs were noted in most of the sections. Elsewhere there was marked proliferation of young connective tissue replacing some of the necrotic muscle. (Figs. 1 and 2.) An unusual atrophy of cardiac muscle without necrosis was seen in 2 of the sections.

Discussion and Conclusions. Myocardial damage can be produced in the dog heart by daily intravenous injections of lanatoside C in doses greater than 0.20 cat unit per kilo of body weight. There may be subendocardial hemorrhage in animals dying after such injections, but the coronary vessels are entirely free of any gross or microscopic abnormalities. These changes probably result from spasm with local lumen attenuation of the coronary vessels supplying the involved areas. Lanatoside C in large doses probably reduces the

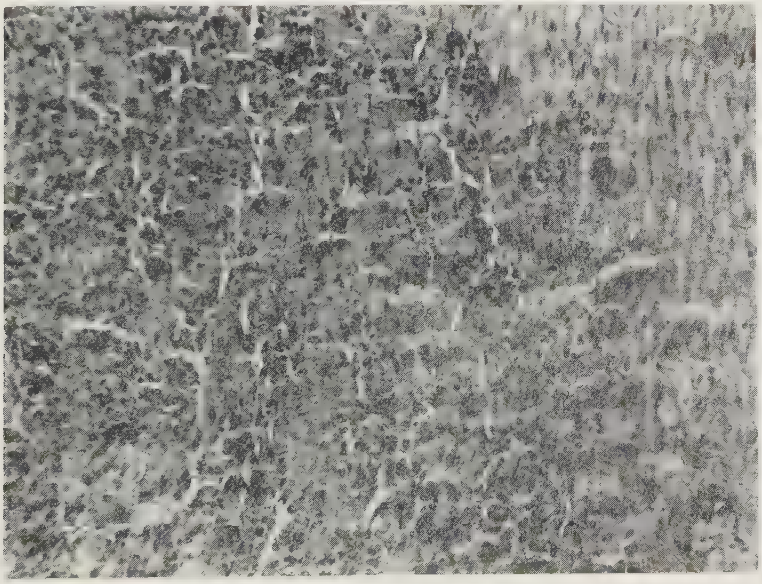


FIG. 1.

Photomicrograph of heart muscle obtained from dog 7E, showing an area of acute myocardial infarction. Note loss of striation of muscle fibers and cellular infiltration. Hemotoxylin-eosin stain was used.

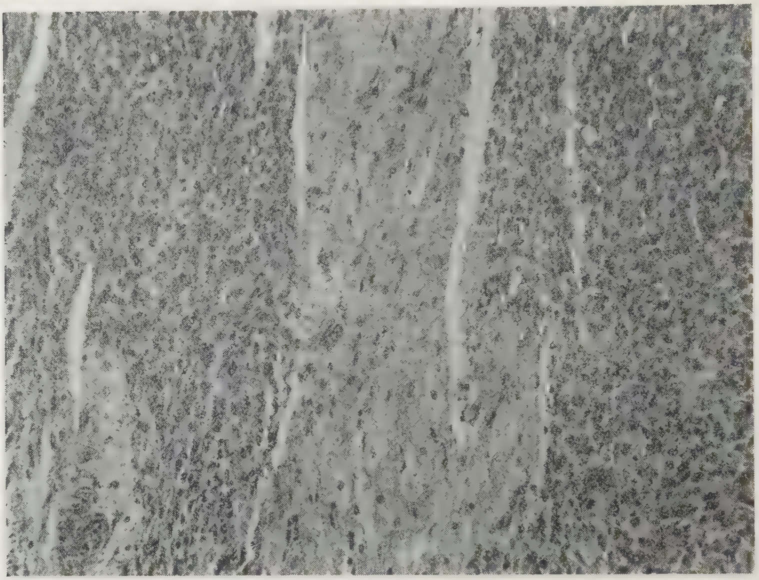


Fig. 2.

Photomicrograph of heart muscle obtained from dog 5E, showing complete replacement of the heart muscle by fibrotic tissue. Note intense cellular reaction. Hemotoxylin-eosin stain was used.

blood supply of the heart muscle by a powerful vasoconstrictor action on the coronary arteries. Essex and Visscher[2] have shown that lanatoside C in therapeutic doses does not restrict the coronary flow as measured by the thermostromuhr.

The myocardial damage which we produced in our experiments is probably not clinically significant, since daily doses approaching the level needed to produce these changes are never given.‡ The importance of producing myocardial infarction without grossly changing or plugging the coronary arteries is, however, emphasized by this study.

---

[2] Essex, E., Herrick, J., and Visscher, M., *Am. Heart J.*, 1938, **16**, 143.

‡ The single, complete digitalizing dose of lanatoside C in man is 8 cc given intravenously. This is equivalent to a single intravenous injection of 1.33 cc in a 25-pound dog. The digitalizing dose in man is never repeated in the same course of treatment, oral maintenance doses are given thereafter (1 to 5 tablets per day).

12095

Growth and Graying of Rats with Total "Filtrate Factor" and with Pantothenic Acid.*

Gladys A. Emerson and Herbert M. Evans.
From the Institute of Experimental Biology, University of California, Berkeley, California.

Rats maintained on a purified ration supplemented with thiamin, riboflavin and pyridoxin fail to grow and solid colored and hooded rats exhibit a characteristic graying of the hair (achromotrichia).

A number of source materials (liver, rice bran and cane molasses) contain a factor or factors that remain in the filtrate after adsorption of extracts of these materials on fuller's earth. The unadsorbed fraction has been termed "factor II" or the "filtrate factor".

Most investigators are agreed that pantothenic acid has a marked stimulating effect on the growth of rats deficient in the so-called "filtrate factor"; however, some workers have reported a complete cure of or prevention of graying and others have reported no effect on pigmentation.

Experimental. Male rats were placed on diet 820[†] at weaning (21 days) and received in addition 15 μg of thiamin 6 times weekly. At the end of 4 weeks all animals had attained a constant weight. Six animals from different litters were used in each assay and each litter was represented by one control. Hooded or solid colored rats were employed. All animals were then given 6 times weekly in addition to the thiamin, 20 μg of riboflavin and 15 μg of pyridoxin. The test substance was administered with the supplements.

Calcium pantothenate was fed at 3 levels: namely, at 40, 50 and 100 μg daily (except Sunday) (Table I). The gain in weight above the controls for the rats receiving the 3 levels of Ca pantothenate would indicate that 100 μg of Ca pantothenate produces little augmentation in growth above that obtained with 40 or 50 μg. On

---

* Aided by grants from the Research Board and the Department of Agriculture of the University of California, Rockefeller Foundation and Merck & Co., Rahway, New Jersey. Assistance was rendered by the Works Projects Administration, Official Project No. 65-1-08-62, Unit A-5.

The following materials were generously contributed: thiamin, pyridoxin and Ca pantothenate, Merck & Co.; riboflavin, Hoffman La Roche Co.; 92% liver extract, Eli Lilly & Co.; molasses, Pacific Molasses Company.

[†] Diet 820: extracted casein[1] 24, salts (McCollum, No. 185) 4, Crisco 8, cod liver oil 2, sucrose 62.

[1] Halliday, N., and Evans, H. M., *J. Nutr.*, 1937, **13**, 657.

the other hand, the animals receiving the lower levels were completely gray while those receiving 100 μg were stippled—the so-called "salt and pepper" effect[‡]—a finding in agreement with Unna and Sampson[2] and with Dimick and Lepp.[3] Unna[4, 5] found that the daily requirement of pantothenic acid for growth was in the neighborhood of 80-100 μg.

The equivalent of 5 g of liver in the form of a liver filtrate (from which the thiamin, riboflavin and pyridoxin had been removed by

TABLE I.
"Filtrate Factor" Activities of Ca Pantothenate and Other Preparations.

Preparation	Quantity fed 6 × weekly,	Gain in wt above controls 60 days	Graying of rats
	μg	g	
Ca pantothenate	40	57	Yes
	50	50	,,
	100	62	Stippled
Liver filtrate	equivalent of 5 g liver	95	No
Liver filtrate + Ca pantothenate	equiv. of 5 g liver + 100 μg Ca pantothenate	92	,,
Ether extr. from cane molasses	equiv. of 3 g molasses	64	,,
Chloroform extr. from ether extr.	equiv. of 3 g molasses	4	Yes
Inositol	1 mg	13	,,
Ca pantothenate	100μg	67	Stippled
Inositol + Ca pantothenate	1 mg inositol + 100 μg Ca pantothenate	69	,,
Choline hydrochloride	1 mg	0	Yes
Choline hydrochloride + Ca pantothenate	1 mg choline hydrochloride + 100 μg Ca pantothenate	69	Stippled

---

[‡] Two gray rats were given 100 μg of Ca pantothenate daily for 4 months. After a period of 2 months a "salt and pepper" appearance was noted which persisted despite continued feeding of the Ca pantothenate.

[2] Unna, K., and Sampson, W. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 309.

[3] Dimick, M. K., and Lepp, A., *J. Nutr.*, 1940, **20**, 413.

[4] Unna, K., *J. Nutr.*, 1940, **20**, 565.

[5] Unna, K., *Am. J. Med. Sci.*, 1940, **200**, 848 (Soc. Proc.).

multiple adsorptions on fuller's earth) stimulated growth to a greater extent than did 100 μg of Ca pantothenate. The rats receiving the liver filtrate were completely protected against graying.

We have shown previously[6, 7] that a preparation obtained from molasses by continuous extraction with ether§ from acid solution both stimulated growth and prevented graying. The residue from this extraction stimulated growth but did not prevent graying. We have frequently observed graying (stippling or definite patterns) in animals receiving a diet containing 10% yeast. Such rats often show the graying during the period of rapid growth but gradually darken after that time. Rats weighing between 250 and 300 g may still show an outline of this pattern.

It would, therefore, appear that the requirement for pantothenic acid for maintaining normal color of the fur is greater than for growth or that some factor or balance of factors, in addition to pantothenic acid is required. Biotin[9] and p-aminobenzoic acid[10, 11] have been suggested as anti-achromotrichia factors.

Woolley[12] has shown that inositol both stimulates growth and prevents alopecia in the mouse. In a preliminary experiment 1 mg of inositol daily was fed to rats both with and without calcium pantothenate (100 μg) and little effect was noted. (Table.) It is, however, of interest to note that 4 of the 6 filtrate factor free controls died while all animals receiving inositol survived, and although in poor condition the rate of growth was greater than for the remaining 2 controls. Six rats receiving Ca pantothenate grew at the same rate as did 6 rats receiving inositol in addition to the Ca pantothenate and the condition of the fur was essentially the same as for the Ca pantothenate alone.

Diet 820 is sufficiently high in its casein content (24%) to protect against choline deficiency; nor did the addition of choline to

---

[6] Mohammad, A., Emerson, O. H., Emerson, G. A., and Evans, H. M., *Science*, 1939, **90**, 377.

[7] Mohammad, A., Emerson, O. H., Emerson, G. A., and Evans, H. M., *J. Biol. Chem.*, 1940, **133**, 17.

§ A chloroform extract prepared from molasses according to the method employed by Nielsen and coworkers[8] in the isolation of a crystalline material from liver which prevented graying, neither stimulated growth nor prevented graying. The ether extract from which the chloroform extract was prepared was active in both respects.

[8] Nielsen, E., Oleson, J. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **133**, 637.

[9] György, P., and Poling, C. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 773.

[10] Ansbacher, S., *Science*, 1941, **93**, 164.

[11] Martin, G. J., and Ansbacher, S., *J. Biol. Chem.*, 1941, **138**, 441.

[12] Woolley, D. W., *Science*, 1940, **92**, 384.

calcium pantothenate further improve the filtrate factor deficiency picture (Table).

Summary. Calcium pantothenate stimulates growth and prevents pattern graying (but not stippling) in filtrate factor deficient rats. The requirement for growth is less than for the prevention of pattern graying. Black rats receiving Ca pantothenate either prophylactically or curatively have stippled fur.

A liver filtrate stimulates growth to a greater extent than does Ca pantothenate and completely protects against graying. It would thus appear that pantothenic acid does not completely replace liver filtrate.

A concentrate prepared by the continuous extraction of cane molasses with ether both stimulated growth and prevented graying while a chloroform extract prepared from it was without activity.

Inositol added to Ca pantothenate does not significantly improve the results obtained with calcium pantothenate alone.

Rats maintained on a 24% casein diet apparently have no need for choline.

12096

Course and Chemotherapy of Experimental Relapsing Fever in the Chinese Hamster (*Cricetulus griseus.*)

Y. P. Chen and Hamilton H. Anderson.

From the Department of Pharmacology, Peiping Union Medical College, Peiping, China.

Many attempts have been made to develop in laboratory animals experimental relapsing fever of sufficient intensity and duration to permit immunologic and therapeutic studies. While monkeys and rodents are susceptible, different strains of relapsing fever spirochetes cause considerable variation in the reaction of the host. Most spirochetes produce infections of short duration in rats, mice and guinea pigs. One exception is *S. sogdianum,* a rare spirochete, which produces in guinea pigs a disease characterized by 2 to 4 relapses.[1]

While the treatment of relapsing fever usually is successful with the arsphenamines many strains of spirochetes have not responded satisfactorily.[2–5] Moreover, spirochetes may disappear from the peripheral blood and remain in the brain and other tissues after therapy. Buschke and Kroó[6] first emphasized the occurrence of residual infection and their observations have been confirmed by others.

---

[1] Sautet, J., *Marseille-Méd.*, 1937, **74**, 273; from *Trop. Dis. Bull.*, 1938, **35**, 499.

Since the chemotherapy of experimental relapsing fever cannot be studied properly unless the course of infection permits adequate treatment and follow-up an attempt was made to develop a suitable infection in a small laboratory animal. We were fortunate in obtaining such an infection with ease in the Chinese hamster *(Cricetulus griseus)* by a California strain of spirochetes transmitted by *Ornithodorus hermsi*—Wheeler.* This strain is more satisfactory than others in Chinese hamsters since the disease is characterized by typical relapses in these animals. Intraperitoneal inoculations of blood from infected mice or hamsters into stock hamsters were invariably successful when 0.15 or 0.2 cc of a dilution of 1 part of tail blood in 9 parts of an aqueous solution containing 0.9% sodium chloride and 2% sodium citrate were used. The infection can be maintained without difficulty in hamsters.

When inoculations were made from animals of one species to those of another the course of infection differed from that observed in animals infected by others of the same species. In 20 mice inoculated with mouse blood the incubation period varied from 22 to 72 hours. Infections were most intense from the second to the fifth day and then gradually decreased with no definite relapses. This is in agreement with Beck's findings.[7]

In 53 hamsters the incubation period varied from 24 to 72 hours. When inoculations were made with mouse blood the incubation period was somewhat shorter, and spirochetes appeared in the peripheral blood in from 24 to 48 hours. There were 3 or 4 attacks characterized by spirochetemia each of from 4 to 6 days' duration with intervals usually of 24 hours. The intensity of infection was

---

[2] Kritschewski, J. L., *Klin. Woch.*, 1927, **6**, 441; from *Trop. Dis. Bull.*, 1927, **24**, 689.

[3] Dickinson, P. S., *Z. f. Hyg. u. Infektionskr.*, 1932, **113**, 683; from *Trop. Dis. Bull.*, 1932, **29**, 564.

[4] De La Camara, P., Fernandez Martinez, J., de Buen, E., and Juarez, E., *Medicina Paises Cálidos*, 1932, **5**, 218; from *Trop. Dis. Bull.*, 1933, **30**, 10.

[5] Francis, E., *Public Health Rep.*, 1938, **53**, 2220.

[6] Buschke, A., and Kroó, H., *J. A. M. A.*, 1923, **80**, 515.

* This strain of spirochetes was supplied through the kindness of Dr. C. M. Wheeler, Hooper Foundation for Medical Research, San Francisco, California.

[7] Beck, M. D., *J. Infect. Dis.*, 1937, **60**, 64.

greater than in hamsters infected with hamster blood and the infection remained usually from 22 to 26 days. Fatalities in the mouse blood infected group appeared less frequently; one-fifth of these hamsters died in from 12 to 37 days. In animals infected with hamster blood spirochetes appeared later, generally from 48 to 72 hours after inoculation and 3 relapses commonly occurred. More than half of the hamsters infected with blood from other hamsters died in from 9 to 30 days, usually after 2 weeks. Brains removed from untreated animals whose blood had been cleared of spirochetes for from 8 to 10 days revealed spirochetes when sections were stained according to the method of Steiner[8] (Fig. 1).

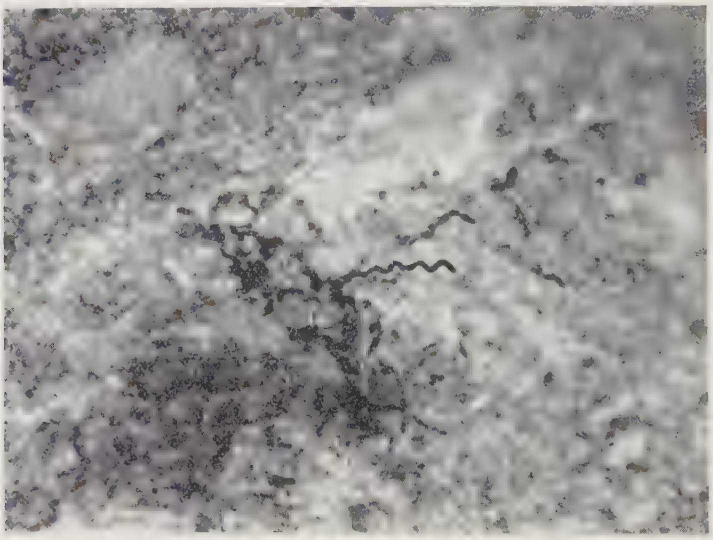


FIG. 1.
Photomicrograph of a section of the brain of an untreated Chinese hamster showing residual infection. Spirochetes are seen in the cortical gray matter. Steiner's stain. × 1940.

Since arsphenamine was first reported by Ehrlich and Hata to be effective against European strains of relapsing fever spirochetes in mice many conflicting reports have appeared.[9] Residual brain infection and so-called "arsenic-fast" strains presumably have accounted for unsuccessful therapeutic trials. In view of these conflicting reports a study of the chemotherapy of experimental relapsing fever in Chinese hamsters was attempted. Two drugs were considered: neoarsphenamine† and a less toxic arsenical, the trisodium salt of 4, 4′ dihydroxy arsenobenzene-N, N′-dimethylene sulfonic acid (Trisodarsen).‡ The LD_{50}, the dose which kills 50% of the animals, of these agents was determined, according to the method of Gaddum, *et al.*,[10] so that a therapeutic dose level might be established. Acute toxicity tests were made on 82 inbred white mice and 54 stock hamsters and chronic toxicity tests were made on 30 mice.

---

[8] Quoted by Spielmeyer, von W., *Technik der Mikroskopischen Untersuchung des Nervensystems*, p. 160, Julius Springer, Berlin, 1930.

[9] Quoted by Fischl, V. und Schlossberger, H., *Handbuch der Chemotherapie*, p. 488, Fischers Mediz. Buch., Leipzig, 1934.

The acute subcutaneous LD_{50} for neoarsphenamine was found to be 235 mg/kg for mice and 190 mg/kg for hamsters. LD_{50} for trisodarsen in hamsters was 685 mg/kg. In chronic toxicity tests, 2 deaths occurred in mice after administration of an amount equivalent to LD_{50} had been given in divided doses over 10 days; no deaths occurred after equivalent amounts of trisodarsen. For treatment, 1/5 of the LD_{50} of each drug was given subcutaneously on alternate days beginning within 72 hours after the animals were inoculated. This dose was given 5 times. Examinations of the tail blood were made daily by means of thin smears prepared with Wright's stain.

A group of 30 hamsters was infected with mouse blood and another group of 30 with blood from other hamsters. Half of the hamsters of each group were treated with neoarsphenamine and the other half were given trisodarsen. In mouse blood infected hamsters given neoarsphenamine, 7 of 15 were still positive 46 hours after the second injection. Four-fifths of the LD_{50} of this drug had to be given before all animals had negative blood smears. Four hamsters developed residual infections, as shown by animal inoculations, and 2 of 15 in this group died.

Animals infected with hamster blood were rid of their spirochetes within 46 hours after the second treatment had been given. After the conclusion of therapy, however, 5 neoarsphenamine treated hamsters of this group showed residual infection by animal inoculations. Three of the 15 hamsters died within 19 days.

Hamsters infected with mouse blood responded promptly to trisodarsen; all showed no spirochetes in the peripheral blood 46 hours after the second treatment. There were 3 residual infections, as shown by animal inoculations, in this group. Two of the 15 animals died. Hamster blood infected animals responded similarly to trisodarsen in that all became negative for spirochetes after the second injection and remained free during the period of observation. Two of 15 died within 19 days. This was the only group that showed no residual infections as shown by animal inoculations.

---

† Purchased locally; prepared by the Abbott Laboratories, North Chicago, Illinois, U.S.A.; Lot No. 905L004.

‡ Supplied through the courtesy of the Abbott Laboratories, North Chicago, Illinois, U.S.A.; Lot No. 811L073.

[10] Gaddum, *et al.*, Med. Res. Counc., Spec. Rep. Ser. No. 128, 1929, and No. 183, 1933.

Three procedures were utilized for confirming the results of treatment: sub-inoculation into test hamsters, examination of brain tissue by Steiner's method[§] and culture on developing eggs after the method of Oag[11] and Bohls, *et al.*[12, ¶] The results of these follow-up studies showed that 9 of the 30 hamsters treated with neoarsphenamine retained viable spirochetes in the brain as revealed by animal inoculations, while only 3 residual infections were found by this test in 30 animals after treatment with trisodarsen. Eight of 15 untreated hamsters showed residual infection by animal inoculations.

Summary. An infection with a California strain of relapsing fever spirochetes was established in Chinese hamsters which responded more satisfactorily to trisodarsen than to neoarsphenamine.

12097

Low Temperature and Radiosensitivity of Skin of New-Born Rats. II. Resistance at Different Dosages.*

T. C. EVANS, W. A. ROBBIE, J. P. GOODRICH AND J. C. SLAUGHTER.
(Introduced by J. H. Bodine.)

From the Departments of Radiology and Zoology, State University of Iowa.

In a previous paper[1] it has been reported that lowering the temperature of new-born rats during irradiation (1,300 r) decreased the amount of injury produced in the skin. It was the purpose of this investigation to determine the amount of this resistance in terms of dosage, and to determine whether or not the resistance would be evident at high as well as at low dosages.

[§] We wish to express our thanks to Dr. Y. K. Hsu, Division of Neurology and Psychiatry, for the staining and examination of brain sections and for the photomicrograph.

[11] Oag, R. K., *J. Path. and Bact.*, 1939, **49**, 339.

[12] Bohls, S. W., Irons, J. V., and De Shazo, T., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 375.

[¶] We would like to express our gratitude to Dr. K. C. Chen, Department of Bacteriology and Immunology, for making inoculations and for the examination of cultures.

[*] Aided by grants from the Committee on Radiations of the National Research Council, and from the Rockefeller Fund for Research on Physiology of the Cell.

The writers are indebted to Professors J. H. Bodine and H. D. Kerr for their interest and helpful criticisms, and to Professor Emil Witschi who supplied the animals from his rat colony.

[1] Evans, T. C., *J. Roentgenol. and Rad. Ther.*, in press.

A series of one-day rats (albino—Wistar strain) was irradiated with dosages from 300 to 3000 roentgens in steps of 300 r. For each rat irradiated at room temperature (24-32°C), a litter-mate was treated while packed in snow or crushed ice. Each animal was taped, ventral surface up, under a 5x9 mm port in a sheet of lead which was 2 mm thick. The radiation (half value layer of ca. 2 mm al.) was unfiltered except for 2 mm of cardboard, and the intensity was usually 134 roentgens per minute. The animals were placed with the mother after the irradiation and were examined 2 weeks later. Transverse sections were made of irradiated and control regions of both ventral and dorsal skins, and the extent of injury determined. The changes noted were (1) desquamation, (2) epilation, (3) epidermal injury and (4) fibrosis. Four degrees of injury could be recognized in each of the above changes. In order to obtain a quantitative measure of injury, each degree of injury was arbitrarily given a value of 1 unit so that the maximum injury in a given section of skin would be 16 units. The region showing the greatest injury was selected for each skin, and the number of units of injury was determined. The value obtained for the ventral skin was added to that of the dorsal skin so that a maximum total injury of 32 was possible for each animal irradiated. By determining the extent of injury in these arbitrary units the writers were able to obtain a dose-effect curve for room temperature (Curve 1, Fig. 1) and another for the animals irradiated at the low temperature (Curve 2, Fig. 1). The vertical lines drawn through the experimental points represent the standard error of the mean of several determinations on the same slides. The determinations were made by 4 individuals and were made at 3 different times. The determined values for injury appear to have a linear relationship to dosage. These curves are not intended to show the absolute relation between dosage and injury but to serve as a basis for comparing the effects of similar dosages given under the two conditions.

It can be seen, by comparing the slopes of curves *1* and *2* of Fig. 1 that the injury increases more rapidly with dosage at room temperature than at low temperature. It is also to be noted that the threshold dosage is much higher for the low temperature curve than for that at room temperature. It is possible to express the increase in resistance of the animals irradiated at the low temperature in

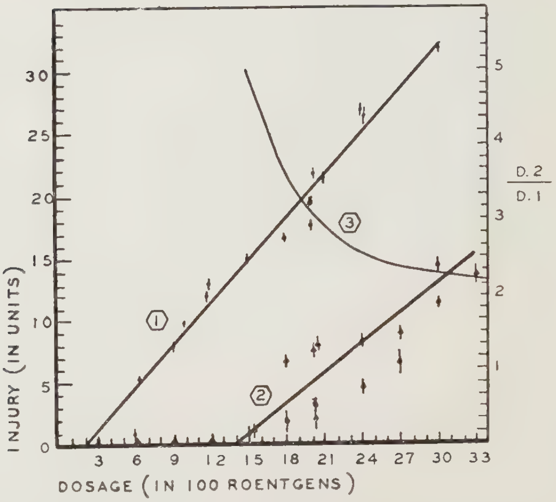


FIG. 1.

Curve 1 is a line drawn, by observation, through determinations of injury produced by irradiating the new-born rats at room temperature (24-32°C). Curve 2 is the result of the same procedure for animals irradiated at low temperature (0-8°C). The ratio of the dosage at low temperature to the dosage at room temperature required to produce the same amount of injury is shown in Curve 3.

roentgens by determining the dosage necessary under the 2 conditions to produce the same degree of injury. For example, a dosage of 2400 roentgens at low temperature gives an injury value of 8 units, and this same amount of injury is produced by only 900 roentgens at room temperature. Therefore, the low-temperature animals at this dosage may be said to be 1500 roentgens more resistant than those treated at room temperature. This difference increases with dosage from 1200 r (at a dosage of 1500 r—cold) to 1700 r (at a dosage of 3300 r—cold). The ratio of the dosage necessary to produce a certain degree of injury in low-temperature animals to the dosage which produces the same amount of injury in room-temperature animals is shown in curve *3*. It can be seen that the ratio decreases rapidly at first and then more slowly as the dosage is increased.

Conclusions. Lowering the temperature of new-born rats during irradiation increased the resistance of the skin so that a dosage increase of 1200-1700 r was required to produce an injury comparable to that of animals rayed at room temperature. The ratio of dosage required at low temperature to the dosage at room temperature decreases from 5 (at 1500 r—cold) to 2.2 (3300 r—cold).

12098

Complement Fixation in "Q" Fever.

IDA A. BENGTSON.

From the National Institute of Health, U. S. Public Health Service, Washington, D.C.

Agglutination tests in "Q" fever have been described by Burnet and Freeman,[1, 2, 3] by Smith, Brown and Derrick,[4] and by Bengtson.[5, 6] In the present work complement fixation in "Q" fever has been investigated to determine whether this test might also be of value in tests for diagnosis and immunity. Such a test has been previously described by Castaneda[7] for Mexican typhus.

Material and Methods. The sera employed were from recovered "Q" fever cases and from recovered infected guinea pigs. The human sera tested were from cases involved in a recent laboratory outbreak[8] and from normal individuals employed in the same building. The guinea pigs were animals used in the routine passage of the American and Australian "Q" fever strains, the American strain being the X-strain described by Dyer[9] and the Australian strain was the strain received from Dr. Burnet, referred to in the publication of Dyer.

Preparation of Antigens. Antigens were prepared from spleens of mice infected by the method of Burnet and Freeman and from yolk sacs infected by the method of Cox.[10] The mouse spleen material was from mice several passages removed from the first inoculation of mice with guinea pig virus, or from mice which had been inoculated with heavily infected yolk sac. These spleens contained numerous rickettsiae. The yolk sac material was from eggs in the 5th or 6th passage of the virus when innumerable rickettsiae were present.

The most suitable antigens were apparently obtained from the material showing the greatest number of rickettsiae. The mouse material was found to give very satisfactory results in the tests with

---

[1-3] Burnet, F. M., and Freeman, M., *Med. J. Austral.*, 1937, **24**, 299; 1938, **25**, 296, 1114.

[4] Smith, D. J. W., Brown, H. E., and Derrick, E. H., *Med. J. Austral.*, 1939, **26**, 13.

[5-6] Bengtson, Ida A., *Pub. Health Rep.*, 1941, **56**, 272, 327.

[7] Castaneda, M. R., *J. Immunol.*, 1936, **31**, 285.

[8] Dyer, R. E., Topping, N. H., and Bengtson, I. A., *Pub. Health Rep.*, 1940, **43**, 1945.

[9] Dyer, R. E., *Pub. Health Rep.*, 1938, **53**, 2277.

[10] Cox, H. E., *Pub. Health Rep.*, 1938, **53**, 2241.

guinea pig serums but was not as satisfactory with human sera. The yolk sac antigens were therefore employed.

The method of preparation was as follows: The yolk sacs of 6 to 8 eggs were removed at the time when the embryos were beginning to die, usually about the 3rd day after inoculation. After draining for a few minutes the yolk sacs were macerated with sterile alundum and suspended in 0.85% sterile saline to a concentration of 10%. This was centrifuged lightly in the horizontal centrifuge to precipitate coarse particles. The supernatant fluid was then centrifuged in the angle centrifuge at 4000 r.p.m. for one hour. The precipitate was removed and suspended in 0.85% sterile saline to the original volume, the supernatant fluid being retained to test for presence of antigen. The suspension of rickettsiae was allowed to stand in the refrigerator for a week or longer, during which time more tissue precipitated, leaving the rickettsiae in suspension, with little tissue remaining. The Australian strain of "Q" fever was used in the experiments reported here.

A titration of the antigen prepared as described, for the complement fixing capacity, was carried out with a known positive guinea pig and human sera. Titrations were also made of the same antigen steamed for 30 minutes in the Arnold sterilizer, and of the supernatant fluid from the high speed centrifugation as well as a Berkefeld-N filtrate of the supernatant fluid.

A 4 plus fixation was obtained with dilutions of the rickettsial suspension up to ⅛. Heating in the Arnold sterilizer had the effect of reducing the results 100%, a 4 plus fixation being obtained with dilution ½ and ¼, but not ⅛. No fixation whatever was obtained with the supernatant fluid from the high speed centrifugation or the Berkefeld filtrate of this fluid. The supernatant fluid and Berkefeld filtrates of the mouse spleen material on the other hand at times gave as good fixation as the suspension of the rickettsiae in the tests with guinea pig serum.

The Test. Guinea pig complement was titrated on the day of the test. The amboceptor was diluted to contain 2 units in 0.2 cc and equal amounts of amboceptor dilution and a 5% suspension of sheep's red blood cells were mixed. The sera to be tested were inactivated at 56°C for 30 minutes and dilutions prepared with sterile saline, ranging from ½ to 1/128. The serum dilutions in 0.2 cc amounts were mixed with 2 units of complement contained in 0.2 cc and with 0.2 cc of the antigen appropriately diluted. Serum controls without antigen were included using dilutions of ½ and ¼ in all tests and in certain tests all dilutions up to 1/128. The mixtures were incubated for 1 hour in a 37°C water bath and 0.4 cc of sensitized cells then added and incubation continued for another hour followed by overnight storage at refrigerator temperature. Controls on the hemolytic system, the antigen, and sensitized cells were included in each test.

Results. The results obtained in tests with 13 human sera from 10 individuals, including controls and 5 guinea pig sera are shown in Table I. The antigen was diluted ¼.

In Table II are shown the results of a test for specificity, using sera from guinea pigs recovered from endemic typhus, European typhus, and Rocky Mountain spotted fever. A serum from a recovered human endemic typhus case not shown in the table gave negative results.

Discussion. The test as carried out gave clear-cut results. Complement-fixing antibodies were present in both human and animal sera as early as 9 days after the beginning of fever in guinea pigs and 13 days after onset in man. The titer of the sera increased in 22 to 23 days and was still higher in the case of one of the human sera tested in 32 days when it reached a titer of 1/128. A titer of 1/32 was present in 285 and 305 days after onset in 2 of the human cases.

TABLE I.
Complement Fixation with Human and Guinea Pig Sera.

Sera	Days after onset	Dilutions of sera							Serum controls			
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:2	1:4	1:8	1:16
Human												
AKa*	13		1	4	4	2	0	0		0	0	0
AKb*	23		3	4	4	3	1	0		0	0	0
AKc	32	4	4	4	4	4	4	3	0	0		
RRa*	48		4	4	4	2	1	0		4	4	1
RRb	307	4	4	4	3	2	0	0	0	0		
LB	285	4	4	4	4	2	1	0	0	0		
CP	305	3	3	3	3	3	1	0	0	0		
5 Normal (?)†		2	1	0	0	0	0	0	0	0		
6 " "		3	3	3	0	0	0	0	0	0		
7 " "		4	3	3	1	0	0	0	0	0		
8 Normal		0	0	0	0	0	0	0	0	0		
9 "		0	0	0	0	0	0	0	0	0		
10 "		0	0	0	0	0	0	0	0	0		
Guinea pig												
1	9	3	3	3	2	1	0	0	0	0		
2	22	4	4	4	4	4	4	1	0	0		
3	31	4	4	4	4	4	4	1	0	0		
4	60	4	4	4	4	4	4	0	0	0		
5 Normal		0	0	0	0	0	0	0	0	0		

*Sera stored at ice box temperature for 1 month or longer which were treated by the Sachs[11] method to remove anticomplementary substances.

† Nos. 5 to 7, inclusive, from individuals engaged in studying "Q" fever.

---

[11] Sachs, H., *Berl. Klin. Wchnschr.*, 1921, **58**, 1075.

TABLE II.
Test for Specificity.

Infection	No. of guinea pig	Days after onset of fever	Dilutions of sera 1:2	1:4	1:8	1:16	1:32	1:64	Serum controls (No antigen) 1:2	1:4
Endemic typhus	1	10	0	0	0	0	0	0	0	0
	2	73	0	0	0	0	0	0	0	0
European typhus	3	10	0	0	0	0	0	0	0	0
	4	60	0	0	0	0	0	0	0	0
Rocky Mountain spotted fever	5	40	0	0	0	0	0	0	0	0
	6	105	0	0	0	0	0	0	0	0
"Q" fever	7	11	2	2	2	1	0	0	0	0
	8	44	4	4	4	4	4	2	0	0

The specificity of the test is indicated by the results obtained with sera from guinea pigs inoculated with European typhus, endemic typhus, and Rocky Mountain spotted fever, no fixation being present in dilutions as low at ½. The tests were made as early as 10 days following beginning of fever and as late as 105 days after.

The sensitivity of the test is indicated by results obtained with the sera of 3 workers who were engaged in the study of "Q" fever. None had suffered an apparent infection and it may be that a certain immunity was established in the handling of the infected material. On the other hand, all had received repeated inoculations of Rocky Mountain spotted fever vaccine prepared from infected ticks, and it is possible that certain lots of vaccine may have been made from ticks simultaneously infected with Rocky Mountain spotted fever and "Q" fever.

The superiority of the yolk sac antigen over the mouse spleen antigen in the tests on the human sera may be accounted for by the greater number of rickettsiae present, but it is also probable that the antigenic substance was more thoroughly separated from the suspension in the case of the yolk sac than the mouse spleen since, as has been pointed out, negative results were obtained with the supernatant fluid of the yolk sac material and positive results with the supernatant fluid of the mouse spleen material.

Summary. A complement-fixing reaction for "Q" fever which is specific among the rickettsial diseases is described. Yolk sac antigen was superior to mouse spleen antigen in tests with human sera. The test was shown to have a good degree of sensitivity and it appeared 9 to 13 days after onset of the disease and persisted for at least 305 days. The indications are that the test is of value for diagnosis and that it affords evidence of immunity.

12099 P

Mating Behavior Induced in Hypophysectomized Female Rats by Injected Estrogen.*

Josephine Ball. (Introduced by R. K. Burns, Jr.)

From the Henry Phipps Psychiatric Clinic, Johns Hopkins Medical School, and the Department of Embryology, Carnegie Institution of Washington, Baltimore.

Five hypophysectomized female rats were given subcutaneous injections of 250 I.U. Progynon-B Schering† (estradiol benzoate) on 5 successive days beginning one week after operation. All of them accepted vigorously aggressive males after a latent period of 2 to 3 days. This is only a low degree of sexual excitability[1] but no higher degrees have been elicited in this laboratory with estrogen alone in castrated females of this strain of rats.[2]

Examination of the sella with a binocular microscope revealed no residual tissue fragments in 3 of these animals. In one of the others there was a little unidentified material in this space and in the fifth animal a remnant of the pituitary gland judged to be posterior lobe was found. The records of the 2 latter were discarded.

In at least 3 of these rats, then, heat behavior had been induced in the absence of anterior lobe tissue. These positive cases render untenable the hypothesis that estrogens may produce heat behavior only indirectly by their effect on the pituitary as previously suggested[3] on the basis of 3 negative cases. Because of the deleterious effect of hypophysectomy on the organism a negative response may be non-specific, whereas a positive response is conclusive. Furthermore, Astwood and Dempsey,[4] have confirmed the above findings, adding many more cases as well as further data to this preliminary study of the interrelations of the hormones producing sex behavior.

---

* This work was supported in part by a grant from the Committee for Research in Problems of Sex, National Research Council.

† Grateful acknowledgment is made to the Schering Corporation for the Progynon-B used in this experiment.

[1] Ball, J., *Comp. Psychol. Monog.*, 1937, **14**, 1.

[2] Ball, J., *J. Comp. Psychol.*, 1939, **28**, 273.

[3] Ball, J., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 416.

[4] Astwood, E. B., and Dempsey, E. W., personal communication.

13000

Relation of Size of Litter to AP Lactogen Content of Nursing Rabbits.*†

J. Meites, A. J. Bergman and C. W. Turner.

From the Department of Dairy Husbandry, University of Missouri, Columbia.

Selye[1] advanced the theory that the maintenance of lactation was due to the act of nursing or milking. Ingelbrecht[2] further contributed to this theory. Reece and Turner[3,4,5] noted a marked decrease in the lactogen content of the AP in rats following the nursing stimuli either with or without milk removal. Holst and Turner[6] confirmed these observations in both guinea pigs and rabbits. From these varied observations it appeared that the nervous stimulation of nursing caused the discharge of lactogen and in turn influenced the maintenance of lactation.

The question arose whether the extent or intensity of milking or nursing would affect the level of lactogen in the AP during the course of the lactation period. Specifically, it was interesting to determine the effect of the number of young in the litter nursing the mother upon the average lactogen content of the pituitary. If the number in the litter were reduced to 2, there would be present a number of mammary glands in which milk stagnation, resorption and involution would be in progress. Would such a condition tend to suppress the lactogen secretion of the AP?

Experimental. As a control, a group of 11 similar lactating rabbits (New Zealand White) were permitted to keep their entire litters of from 5 to 11 young. The experimental group of 10 lactating animals had their litters reduced to 2 on the fifth day postpartum. The mothers of both groups were sacrificed on the 20th day postpartum. The pituitaries were removed and assayed quantitatively for lactogen by the Reece-Turner method.[5]

---

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 733.

† This study has been aided in part by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

[1] Selye, H., *Am. J. Physiol.*, 1934, **107**, 535.

[2] Ingelbrecht, P., *Compt. rend. Soc. de Biol.*, 1935, **120**, 1369.

[3] Reece, R. P., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 367.

[4] Reece, R. P., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1937, **35**, 621.

[5] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 266, 1937.

[6] Holst, S., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 479.

TABLE I.
Relation of Size of Litter to the AP Lactogen Content of Nursing Rabbits.

Group	No. of rabbits	Body wt avg, g	Pituitary wt avg, mg	Units per pituitary		Units per mg pituitary tissue		Units per 100 g body wt	
				R.-T.*	I.U.*	R.-T.	I.U.	R.-T.	I.U.
Control	11	3360	42.20	30.05	1.35	.71	.032	.89	.040
Exp.	10	3617	41.03	27.10	1.22	.66	.030	.75	.034

*1 International Unit = 22.2 Reece-Turner units (Meites, J., Bergman, A. J., and Turner, C. W., *Endocrinology*, 1941, **28**).

It will be seen that there is an average of 30.05 Reece-Turner units or 1.35 I.U. of lactogen per pituitary in the control rabbits which were allowed to keep their entire litter. Holst and Turner,[6] using a similar group of 4 lactating rabbits with entire litters, found an average of 26.63 Reece-Turner units or 1.20 I.U. per pituitary. In the experimental group of rabbits, with litters uniformly reduced to 2 young, there was an average of 27.10 Reece-Turner units or 1.22 I.U. per pituitary. Since the difference between these 2 groups is too small to be significant, it is concluded that the size of the nursing litter does not influence the lactogen content of the pituitaries of nursing rabbits.

Summary. The lactogen content of the AP was quantitatively determined in 21 lactating rabbits killed on the 20th day postpartum, No significant difference was found in hormone content between the 11 does which were permitted to keep their entire litters of 5 to 11 young, and the 10 does whose litters were uniformly reduced to 2 on the 5th day postpartum.

13001 P

Effect of 1(—)-Tyrosine on Liver Glycogen of the Normal Rat.*

JOSEPH S. BUTTS, RUSSELL O. SINNHUBER AND MAX S. DUNN.

From the Department of Chemistry, Oregon State College, Corvallis, and the Chemical Laboratory, University of California at Los Angeles.

In an earlier publication[1] the effect of feeding *dl*-phenylalanine and *dl*-tyrosine on the liver glycogen of rats was described. The former compound gave rise to an appreciable amount of glycogen in the liver, whereas the hydroxy acid failed to elicit a significant response. In this communication the effect of feeding the naturally occurring isomer of tyrosine on liver glycogen formation is reported.

---

* This study made possible by the support of a grant from the American Philosophical Society.

[1] Butts, J. S., Dunn, M. S., and Hallman, L. F., *J. Biol. Chem.*, 1938, **123**, 711.

Female rats ranging from 130-180 g in weight were used in this study. They were subjected to a 48-hour fast preceding the feeding of the tyrosine. Filter paper was placed in the cages as it was found that this would be eaten and cause bulk, thereby decreasing the chances of diarrhea. As demonstrated earlier[2] this ingested paper had no effect on glycogen formation.

The insolubility of the tyrosine precluded the possibility of feeding it as the free acid. Since neither the hydrochloride nor the sodium salt is well tolerated in fairly large amounts, a suspension of the 1(—)-tyrosine was prepared as follows: the tyrosine was ground to a fine powder, suspended in a 1.75% aqueous gum tragacanth medium in a proportion of 10 g of the acid to 100 cc of the medium, giving a uniform suspension readily administered by stomach tube. To avoid a large excess of the tyrosine at any one time, the animals were fed approximately 200 mg every 2 hours. The control animals were similarly fed 2 cc of the gum tragacanth suspension containing 5 g sodium chloride per 100 cc of the suspension.

At the end of 3, 6, 9, and 12 hours, groups of animals were sacrificed, under amytal anesthesia, the livers removed and analyzed for glycogen according to the method of Good, Kramer and Somogyi.[3]

To prove that an excess of tyrosine had been fed the gastrointestinal tracts were removed, minced, and extracted three times with dilute hydrochloric acid. The washings from each sample were pooled and 10 cc of a 20% trichloracetic acid solution was added. After standing 24 hours the samples were made up to a known volume and filtered. They were then analyzed for amino nitrogen by the Van Slyke procedure. Although the method used did not quantitatively recover the amino acid present, as judged by the recovery of known amounts of tyrosine, it was sufficiently accurate to establish the presence of an excess of the amino acid. The average recovery was 82%, with known amounts of tyrosine. Therefore, it seemed certain that an excess of tyrosine was present and that maximum absorption was occurring at all times.

Table I gives the results of 1(—)-tyrosine feeding on liver glycogen deposition.

---

[2] Butts, J. S., Blunden, H. D., and Dunn, M. S., *J. Biol. Chem.*, 1937, **119**, 247.

[3] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

TABLE I.
Glycogen Content of Livers of Female Rats Receiving 1(—)-Tyrosine.

		Control				1(—)-Tyrosine	
No. of animals	Elapsed time, hr	%	Stand. Dev. of mean	No. of animals	Mg Tyrosine fed	%	Stand. Dev. of mean
15	3	.09	± .015	8	300	.26	± .045
15	6	.09	± .010	8	600	.39	± .016
14	9	.06	± .001	12	800	.67	± .078
16	12	.04	± .006	9	800	.69	± .104

Discussion. In view of the earlier findings that *dl*-tyrosine when fed to a starving rat did not cause an appeciable deposition of liver glycogen it is rather surprising that under comparable conditions 1(—)-tyrosine does cause an increase. One is therefore forced to the conclusion that under similar experimental regimes, the 1(—)-tyrosine quantitatively effects liver glycogen formation in a very different manner from the racemic mixture.

It was noticed that when urine from a rat fed 1(—)-tyrosine was treated with ammonium phosphomolybdate, as in the Briggs[4] method for homogentisic acid, a copious reduction occurred. The animals were fed 4 mg per g of body weight per day. The feeding was continued for 6 days with no other food available during this time. Although less intense reduction occurred when the amount of 1(—)-tyrosine was lowered to 2 mg per g per day a distinct color did develop.

It is not claimed that homogentisic acid alone was responsible for this effect, as it is known that other di-hydroxy compounds cause a similar reduction. Actually there is no proof that homogentisic acid was present, as we were unable to isolate a derivative of this compound. Under similar conditions when *dl*-tyrosine was fed no detectable reduction occurred.

Conclusion. 1(—)-Tyrosine when fed to rats starved 48 hours produces an increase in liver glycogen. This is in marked contrast to the result obtained when *dl*-tyrosine is fed. The urine from rats fed 1(—)-tyrosine caused a reduction of ammonium phosphomolybdate. Under similar experimental conditions *dl*-tyrosine caused no such reduction.

---

[4] Briggs, A. P., *J. Biol. Chem.*, 1922, **51**, 453.

13002

Quantitative Determination of Activity of Streptococcal Fibrinolysin.

L. R. Christensen. (Introduced by L. R. Jones.)

From the Department of Bacteriology, St. Louis University School of Medicine, St. Louis, Mo.

A survey of the literature indicates that very few quantitative studies have been made on streptococcal fibrinolysin. The majority of attempts which have been made have involved serial dilutions of cultures or culture-filtrates which were incorporated in fibrin clots and in this way tested for lytic activity. Van Deventer[1] proposed as a unit of fibrinolysin that amount causing dissolution of a plasma fibrin clot at the end of 24 hours. Fraser and Madison[2] used essentially the same technic with the substitution of fibrin prepared from freshly-prepared fibrinogen and thrombin solutions. Later, Madison and Taranik[3] attempted a still further standardization of the test. In this procedure, the highest dilution causing lysis at the end of 2 hours contained one unit of fibrinolytic activity.

Massell, Mote and Jones[4] in an extensive study of antifibrinolysins devised a method for measuring the amount of antifibrinolysin present in a serum, based on their belief that fibrinolysin is quantitatively neutralized by antifibrinolysin. By appropriate modification of their procedure, it is possible to compare the lytic activity of 2 or more lysins, but absolute quantitative results are not obtained.

Lack of uniformity in the fibrin used as substrate, the effects of various media components, and the effects of other enzymes in the culture filtrates have seemed to render difficult the development of a satisfactory quantitative test. Further, in none of these reports has any data on retests been given, so it is impossible to evalute the reliability of the various procedures from the standpoint of reproducibility. Also, it has been noted in this laboratory that freshly-prepared fibrinogen samples vary in their susceptibility to lysis. It is believed that the chief factor in these differences in susceptibility is the variable protein content of such fibrinogen solutions.

---

[1] Van Deventer, J. K., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 50.

[2] Fraser, F. H., and Madison, R. R., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 307.

[3] Madison, R. R., and Taranik, J. D., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 1.

[4] Massell, B. F., Mote, J. R., and Jones, T. D., *J. Immunol.*, 1939, **36**, 45.

With a standardized, stable substrate available through the use of dried fibrinogen and dried prothrombin,[5, 6] the possibility of the construction of a satisfactory quantitative fibrinolysin test was investigated.

The effects of fibrinogen concentration, temperature of incubation, and pH on fibrinolysis have been discussed in a previous paper,[6] together with the reasons for the use of 0.1% fibrinogen solution in the preparation of the standard fibrin clot. In the experiments to be reported, suitable culture-filtrates, serially diluted in M/10 phosphate buffer (pH 7.2-7.3) were added in 0.2 cc volume to 1.0 cc of the standard fibrinogen solution prepared from desiccated fibrinogen and the mixtures clotted by the addition of 0.1 cc of thrombin solution prepared from dried prothrombin. The fibrin-lysin mixture had a pH of 7.2-7.3, and was incubated at 40°C in a water bath following clotting.

A typical lysis curve is presented in Fig. 1. Lysis time represents the time in minutes elapsing between clotting and complete dissolution of the fibrin clot. It can be seen that over the greater portion of its length the curve approximates a straight line. All lysis curves are of the same shape, although the slope of the curve varies inversely with the original strength of the fibrinolysin sample.

Determination of the Fibrinolytic Unit. Originally, it was decided to designate as one unit that dilution of lysin, obtained by in-

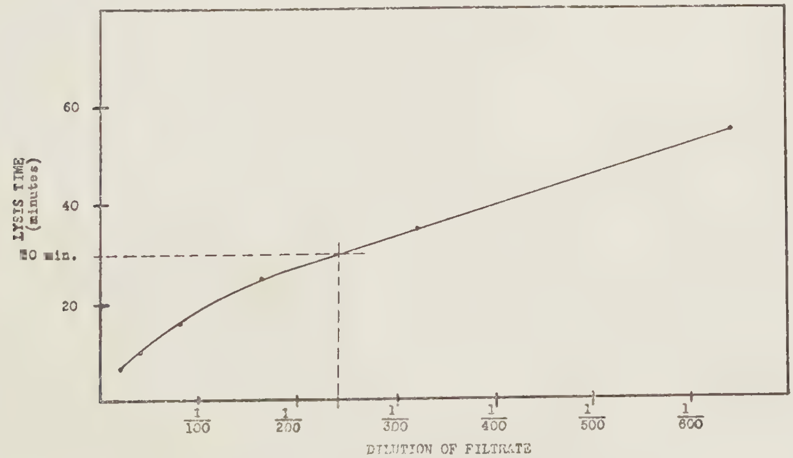


FIG. 1.
Quantitative Determination of Fibrinolysin.

---

[5] Christensen, L. R., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 569.

[6] Christensen, L. R., *J. Inf. Dis.*, 1940, **66**, 278.

terpolation on the lysis curve, which lysed the standard clot in one hour. It was noted, however, that as the incubation period approached one hour, the tubes containing the higher dilutions of fibrinolysin showed marked shred formation in the clot due to denaturation of the fibrin. With experience, the true end-point of lysis could be approximated fairly accurately, but because of this difficulty, a shorter interval, 30 minutes, was adopted as the end-point of the titration. At this time-interval, lysis is more clear-cut, without the marked shredding of the fibrin seen on longer incubation, and by this time the lysis curves have straightened. One unit of fibrinolysin is defined, therefore, as that amount, in a volume of 0.2 cc, which lyses the standard fibrin clot in 30 minutes, this amount being determined by interpolation on the lysis curve. A simple calculation will then give the number of units of lysin in 1 cc of the original material.

The following example will illustrate the method: Serial dilutions of a sample of fibrinolysin obtained from strain 1212 were prepared and tested against standard fibrin clots, with the lysis times recorded in Table I.

When these data are plotted (Fig. 1), it is seen that the dilution lysing in 30 minutes, obtained by interpolation would be 1:240. This dilution contains 1 unit; therefore, 1 cc of the original, undiluted material contains 1200 units (240 × 5).

The unit herein described is admittedly arbitrary and artificial. It does, however, have definite advantages which make it practicable when large numbers of quantitative tests must be made. The test as described is simple to perform, results are available in a short time (30-45 minutes), and the short incubation period precludes growth of the microörganisms influencing the results when whole cultures are tested.

Evaluation of Method. To establish the reproducibility of the method, a batch of fibrinolysin was prepared and stored in the ice box. Experience has shown that if adjusted to the proper pH, such filtrates will show no significant variation in activity over several days. For test materials, batches of dried fibrinogen and of dried prothrombin were used, and successive titrations of the lysin were made over a period of 3 days. In Table II, the number of units was determined by the method outlined above. The figures re-

TABLE I.
Titration of Fibrinolytic Activity in Minutes.

Dilution	1:20	1:40	1:80	1:160	1:320	1:640
Lysis time, min	7	10	15½	25	35	54½

corded in the "fibrinogen" and "thrombin" columns refer to the solution prepared from the dried reagents. Thus, the first result in Table II indicates that the third solution of fibrinogen prepared from the dried material was clotted with the first solution of thrombin prepared from the dried prothrombin.

The first half of Table II represents those tests, selected from the entire series, in which the thrombin and fibrinogen solutions were not over 1 hour old at the time of testing. The remainder of the tests in the table illustrate the influence of the age of the test solutions (fibrinogen and thrombin) on the results. In these latter tests either one or both of the reagents was over one hour old at the time of testing.

From these data, it is quite evident that quantitative determinations are not reliable if either the thrombin or fibrinogen solutions are more than one hour old. However, when freshly-prepared solutions are used, agreement of successive determinations is excellent. It must be emphasized that fibrinogen and thrombin solutions will serve for qualitative tests of lysin activity for many hours, even several days if kept in the ice box. Evidently, however, the slight denaturation which occurs in even a short time exerts a marked influence on lysis time. The nature of the changes occurring in the reagents is not known, but it is of interest that in some cases lysis time is decreased and in others it is increased.

TABLE II.
Titration of Fibrinolytic Activity.

(Fresh reagents)			(Old reagents)		
Fibrinogen	Thrombin	Units	Fibrinogen	Thrombin	Units
3	1	1065	1	2	1185
4	3	1115	1	2	835
5	4	1245	2	1	685
5	4	1200	2	2	860
7	5	1120	3	2	810
9	7	1230	1	2	1435
10	8	1170	2	2	1355
10	8	1170	3	2	1310
11	9	1150	2	1	1575
11	9	1200	4	2	1280
11	9	1175	4	3	1315
12	10	1100	5	4	980
12	10	1075	5	4	930
			7	5	1425
			7	4	1600
			8	6	1600
1155	Mean		1198	Mean	
54.77	Standard Deviation		293.3	Standard Deviation	
4.74%	Coefficient of Variation		24.48%	Coefficient of Variation	

13003 P

Course of Diabetes Insipidus Following Hypophysectomy in the Rat.

E. L. Corey and S. W. Britton.

From the Physiological Laboratory, University of Virginia Medical School.

In earlier studies on the hypophysectomized rat[1, 2] we have been particularly concerned with the phase of acute diabetes which immediately follows pituitary ablation, and continues thereafter for several days. Earlier reports have affirmed the transitory nature of the diabetic condition in the rat.[3, 4, 5] We have now extended our studies of fluid metabolism and urinary chloride excretion to include more protracted periods, up to 80 days after operation. Fluid exchanges were determined in individual metabolism cages fitted with graduated drinking tubes; the urine was collected under toluene in graduated cylinders, and the chloride concentrations determined by means of the Volhard titration.[6] Over 70 individual tests were made, and the results compared with those obtained from 34 normal animals.

Study of the data obtained has led us to divide the post-operative diabetic state seen in the hypophysectomized rat into 3 arbitrarily determined stages: 1, a primary acute condition persisting for 3 to 5 days, in which polyuria predominates and the water-intake urine-

TABLE I.
Course of Diabetes Insipidus and Hypochloruria Following Hypophysectomy.*

Phase of diabetes insipidus	No. of cases	Post-operative period (days)	Avg water intake (100 g rat)	Avg urine output (100 g rat)	Avg urinary chloride (mg/cc)
Primary acute	30	0- 5	6.3	7.1	0.34
Secondary acute	21	5- 8	3.6	2.9	1.14
Tertiary chronic	26	8-72	2.3	1.9	2.12
Normals	34		1.7	1.5	2.78

*All metabolism tests were made over a 12-hour period. When repetitive tests were made on the same animals, 2 to 4 days were allowed for "rest" between runs.

---

[1] Silvette, H., and Britton, S. W., *Am. J. Physiol.*, 1938, **123**, 630.

[2] Corey, E. L., Silvette, H., and Britton, S. W., *Am. J. Physiol.*, 1939, **125**, 644.

[3] Richter, C. P., *Am. J. Physiol.*, 1933, **106**, 80.

[4] Richter, C. P., *Am. J. Physiol.*, 1934, **110**, 439.

[5] Pencharz, R. I., Hopper, J., and Rynearson, E. H., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 14.

[6] Peters, J. P., and VanSlyke, D. D., *Quant. Clin. Chem.*, Williams and Wilkins, 1932.

output ratio (W/U) is less than unity; 2, a secondary (but still acute) phase of about 3 days in which an emerging polydipsia becomes evident and during which water intake exceeds urine output, with an average W/U ratio for the period of more than 1; 3, a tertiary "chronic" condition of mild diabetes in which the hypophysectomized animal shows a slight but constant increased water intake and urine output, with a W/U ratio almost identical with that of normal controls.

These results confirm our own previously published findings and in part those of Richter concerning diabetes insipidus in the hypophysectomized rat. They further indicate that the condition is not "transitory" in nature, but that a mild, chronic diabetic state persists for at least 80 days. The recent observations of Schweizer, *et al.*,[7] that a polyuric state persists in hypophysectomized rats until they become moribund are essentially in agreement with the above. That the alterations in the diabetic conditions described may be correlated with cortico-adrenal and gonadal atrophy may be considered. In this connection it has recently been shown in this laboratory[8] that there is a specific antagonism between desoxycorticosterone and post-pituitary extracts in their effects on water balance. The former substance, in normal as well as adrenalectomized and hypophysectomized rats, produced increases in water intake and urine output and reductions in urinary chlorides, while the post-pituitary principle brought about reverse reactions in all cases.

Summary. The polydipsia, polyuria and hypochloruria which follow hypophysectomy in the rat have been described as primary acute, secondary acute, and tertiary or chronic. The mild, chronic diabetes persisted in these experiments for 80 days and terminated only shortly before death.

---

[7] Schweizer, M., Gaunt, R., Zinken, N., and Nelson, W. O., *Am. J. Physiol.*, 1941, **132**, 141.

[8] Britton, S. W., and Corey, E. L., *Science*, in press.

13004 P

Positive Potentials Recorded from the Superior Colliculus.*

Geo. H. Bishop and James O'Leary.

From the Washington University School of Medicine, St. Louis.

A needle electrode thrust into an active nerve pathway may so kill nerve fibers in its vicinity that one obtains an effective dead-end lead, with a positive potential. When 2 electrodes are effectively on opposite sides of a region of synapses, the endings of fibers at synapses act similarly. In the superior colliculus of the cat one regularly finds that a probe electrode thrust downward encounters a shallow negative post-synaptic potential, following stimuli to the optic nerve, then a deeper positive potential, usually of considerably higher voltage. Records obtained as the electrode is withdrawn may show, on the other hand, a reversal of the initial upper negativity, giving a positive spike at any level. The potentials encountered in the colliculus may be explained by conventional physiological considerations without the inference of a relative positivity at an active region of a tissue.

From the central region of synapses with optic tract fibers, in the colliculus, pathways radiate out in several directions. In the extreme case, of fibers conducting in all directions from a center, one can consider as an element the cone whose base is a small surface area, subtending a solid angle at the center. Since in the symmetrical case activity in elements adjacent to this will prevent current from flowing across the boundary of such a cone, it may be considered as if insulated in air, and for a small surface area, this element approaches a linearly arranged nerve fiber bundle. The critical feature of the solid figure to be retained is that the shunting material between the fibers will increase, and the resistance decrease, as the square of the distance from the center.

The potential distribution along the surface of such an element will be triphasic, and the positive phase toward the center will be of higher amplitude, because less shunted, than the peripheral positivity. From electrodes placed at the center and at the periphery, therefore, an overall central positivity will be recorded, the more so as the peripheral electrode will usually be at some distance from the ends of active fibers.

The negative spike recorded peripherally will be increasingly

---

* Aided by grants from the Rockefeller Foundation, the Scottish Rite Masons Fund and the John and Mary Markle Fund.

shunted, and therefore decrease in recorded amplitude, as the electrode is moved away from the central synaptic region, and the transition from negative to positive phases should be correspondingly abrupt at the central ends of the conducting elements.

Plots of spike potential amplitude downward through the colliculus correspond to this scheme, with the qualification that the distribution of fibers required to account for the results is more hemispherical than spherical, and relatively few elements conduct perpendicularly downwards. Due to the "shading" of the central positive region by the peripheral negative hemisphere, any peripheral reference point tends to be relatively negative, and since the reversal point is merely the point isoelectric with the reference electrode, the configuration of the conducting system is a factor in determining the level of reversal and the positive and negative amplitudes. In the periphery of the colliculus only negative spikes are encountered at any depth. On withdrawing the electrode the deep positivity may be little changed, when the surface negativity is reduced or completely reversed due to local injury of fibers along the needle shaft. (Fig. 1.)

The post-synaptic fibers of the cat colliculus are apparently largely non-myelinated, and part of the second phase following the spike potential is presumably an after-potential, reversing with depth for the same reason as the spike reverses. Part is also, however, assignable to successive neurones, so situated in the lower levels of

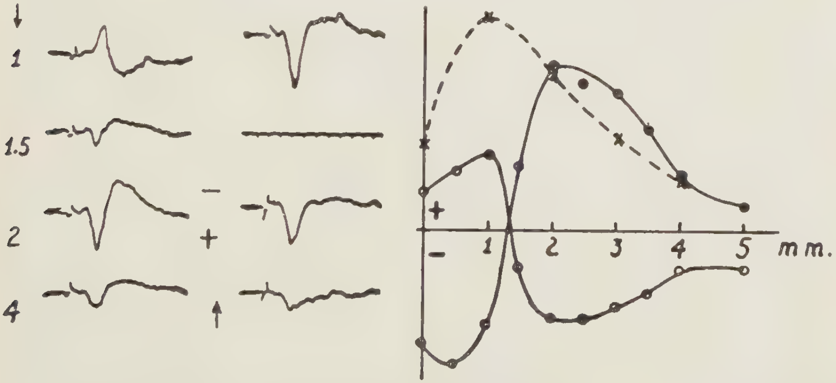


FIG. 1.

Left column, records of colliculus from surface downward to depths indicated, reference electrode in medial geniculate. Right, records from the same depths as needle is withdrawn, all spikes positive. Time 5 msec. intervals. Graph, solid circles, spike amplitude against distance from surface; hollow circles, second phase amplitude; crosses, spike amplitude as needle is withdrawn. Maximal D.C. stimuli to optic nerve in socket. Cat, nembutal 0.25 cc/kg.

the colliculus as to occasion a late negativity there, and a corresponding positivity at upper levels. This will explain certain records of an apparent second-phase potential greater in amplitude than the first phase. Furthermore in the rabbit, where an earlier potential is assignable to larger fibers, at a specific level deep in the colliculus the apparent second phase is replaced by a series of spikes, as occurs in Therman's[1] record from the medulla.

It is obvious that the positive spike may be affected by all activity radiating out from the central region, while the negative spike can be considered to have two components, one the potential assignable to the field in general, like the positive spike, and one assignable to the specific fibers with which the electrode is in contact. If the latter are injured, this component may be reversed in sign, and the record is the algebraic sum of the two components. A corresponding injury in the region of positive potential will, however, affect but a small fraction of the elements responsible for the positivity, with a resulting disparity of positive and negative amplitudes. The reversal may, therefore, occur at any depth, depending on the position of synapses, the degree of injury, and the geometry of the conducting system as a whole.

13005

Water and Electrolyte Metabolism in Diabetes Insipidus.

H. I. CHU, S. H. LIU AND T. F. YU.

From the Department of Medicine, Peiping Union Medical College, Peiping.

In view of the fragmentary information concerning the salt and water metabolism in diabetes insipidus,[1] the following data on the serum protein, water, sodium, potassium and chloride and the urinary output of these elements on known intake in a case of severe but uncomplicated diabetes insipidus may be of interest. The patient was an unmarried Chinese woman of 20 with a history of the disease for 3 years. Various investigations including X-ray of the skull and blood Wassermann reaction gave no clue as to the etiology of the condition. On a regimen of full hospital diet and fluids as desired, her water intake and urine output amounted to 16 liters a day and her weight was maintained at 53 kg. The metabolic results, presented in Fig. 1, may be epitomized as follows:

---

[1] Therman, P. E., *J. Neurophysiol.*, 1941, **4**, 153.

[1] Talbott, J. H., Coombs, F. S., Consolazio, W. V., and Pecora, L. J., *Arch. Int. Med.*, 1940, **66**, 607.

1. *Effect of moderate water restriction and increasing salt intake while on a "solid" diet.* From Dec. 14 to 17, a constant diet containing rice, pork, eggs, vegetables and fruits, totaling 1,300 calories, was served, together with 2 g of sodium chloride. The water intake (distilled water) was limited to 10 liters a day. There was moderately severe thirst, accompanied by a sharp drop in body weight and negative water balance. Serum dehydration was evidenced by a decrease of serum water from 91.8 to 90.5%, and an increase of serum protein from 6.22 to 7.50% on Dec. 16. Serum sodium increased from 141.4 to 151.1, and chloride from 107.9 to 111.6 m. Eq. per liter. Serum potassium remained unchanged. While the urinary sodium and chloride approximately equalled their respective intakes, there was a loss of potassium. When the same regimen was continued, these changes tended to wear off so that by Dec. 18 her control body weight was partially regained, and water balance was maintained, and evidence of hemoconcentration disappeared.

An attempt was made to increase the sodium chloride intake to 4 g per day on Dec. 18-20, but the thirst became intolerable, quantitative consumption of the diet was impossible, and vomiting occurred on several occasions. The partial starvation resulted in marked nitrogen loss and irregular electrolyte intake, but the water balance was maintained and serum composition preserved.

2. *Effect of increasing salt intake while on a liquid diet and constant water intake.* From Dec. 21, a constant liquid diet consisting of rice gruel, orange juice and cow's milk with added sugar (total calories, 1,440) was employed. The total daily fluid intake was made up to 10 liters on which the patient felt comfortable. The sodium chloride intake, starting with 2 g, was progressively increased to 8 g per day. This was well tolerated, but the body weight showed a definite decrease, although the urinary output was not excessive in comparison with the intake. The serum sodium was elevated from 138.9 to 144.5, potassium from 2.98 to 3.98, and chloride from 99.0 to 103.3 m Eq per liter as the sodium chloride intake was progressively increased. The urine sodium and chloride output exceeded their intake only when the salt allowance was 2 g, but fell short of the intake on higher levels of salt allowance. The potassium balance was negative throughout, related partly to the prevailing negative nitrogen balance and partly to a probable loss of intracellular water.

3. *Effect of pitressin.* While on the same regime of liquid

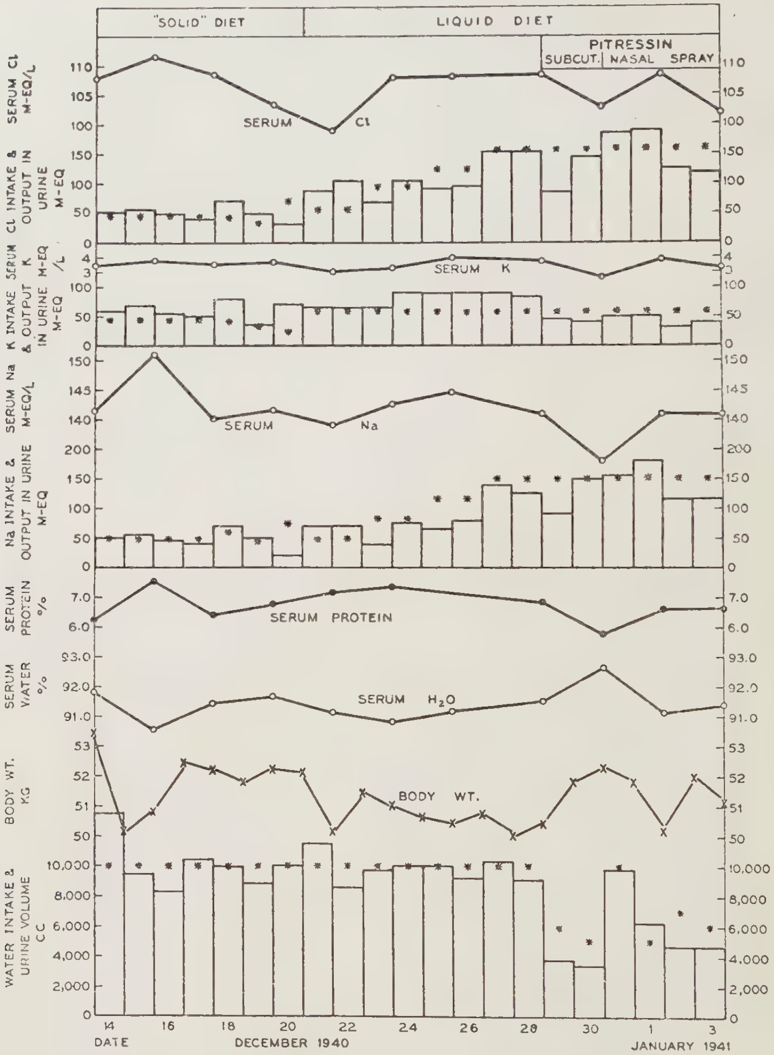


FIG. 1.

Effect of water restriction, varying levels of sodium chloride intake and pitressin on the water, sodium, potassium and chloride metabolism in diabetes insipidus. Asterisks indicate intake and columns output in urine.

diet with 10 liters of fluid and 8 g of sodium chloride, the patient was given 0.5 cc (10 pressor units) of pitressin (Parke, Davis) hypodermically every 6 hours on Dec. 29-30. Immediately the urine output was reduced to 3360-3680 cc, and the water intake voluntarily decreased to 5000-5900 cc, giving a positive water balance as well as an increment in body weight. There were unmistakable signs of hemodilution. Plasma volume increased from 2060 cc (Dec. 29) to 2480 cc (Dec. 31), serum water from 91.5 to 92.6%, serum protein decreased from 6.84 to 5.77%, and hematocrit from 40 to 35%. Serum sodium fell from 140.9 to 133.0 m Eq per liter. Likewise there was a fall in serum potassium and chloride. Sodium and chloride balances were positive on the first day of pitressin injections, and potassium balance was positive throughout. Nitrogen balance became less negative.

When pitressin in the same dosage was given as nasal spray on Dec. 31-Jan. 3, the result on the first day was not satisfactory because of improper administration of the drug, but the data on the subsequent 3 days indicated that the efficiency of intranasal administration was not definitely inferior to that of subcutaneous injection.

Summary. The data presented indicate that in diabetes insipidus the serum water and electrolyte concentrations are maintained normal by reason of the unlimited water exchange. Water restriction or sodium chloride ingestion tends to produce negative water balance, hemoconcentration, augmented sodium and chloride levels in the serum, and loss of potassium from the body. Such changes tend to decrease in extent with elapse of time in spite of continued water restriction and salt ingestion. Pitressin rapidly restores the water and electrolyte concentrations of the serum and probably of other body fluids to normal and this is accomplished on a much lower level of water exchange.

13006 P

Effect of Hibernation on Rate of Eruption and Dentin Apposition in the Ground Squirrel Incisor.

BERNARD G. SARNAT AND WALTER E. HOOK. (Introduced by I. Schour.)

From the Department of Histology, College of Dentistry, University of Illinois, and the Department of Medicine, University of Chicago.

No studies have been found on the relationship of hibernation to growing teeth. The purpose of this investigation was to determine the effect of hibernation on the rate of eruption and dentin apposition in the thirteen-lined ground squirrel. This animal was selected because of ready availability, ease of artificial induction of hibernation and continuous eruption and apposition of dentin of the incisor.

Material and Method. This report is based on 9 control and 26 experimental animals (of unknown ages) which were caught in the vicinity of Chicago, Illinois, during the summer of 1940. The animals were kept in the laboratory for 2 months on standard rat ration. Hibernation was induced in the experimental group by withholding food and water for 2 days and then placing the animals in a dark constant-temperature room kept at 2-5°C.

A transverse notch was made at the labio-gingival border. Its distance from the incisal edge was measured by caliper after various periods. These measurements calculated in mm per week were of the rate of attrition which is essentially the same as the rate of eruption.[1] Direct measurements of the rate of eruption were not made because in many experimental animals the distance between the gingiva and the notch was very small.

The rate of dentin apposition was determined by the alizarine red S method.[2] This dye is deposited in the dentin calcifying at the time of injection and can be seen as a sharp red line in the ground section of the tooth. An intraperitoneal injection of a 2% solution of alizarine red S (100 mg/kg) was given to each animal at the beginning and at the end of the experimental and control periods. The skulls of the sacrificed animals were split mid-sagitally and fixed in a 10% neutral formalin solution. Longitudinal ground sections were made of the upper incisors; the distance between the alizarine red S lines was measured by micrometer and the rate of dentin apposition calculated in microns per day.* In the animals which died before the second injection of alizarine red S was given, the measurements were made from the alizarine red S line to the pulpal border of the tooth.

These experiments were conducted during the months of October, November and December of 1940:

Experimental Group (2-5°C). The animals were injected with alizarine red S and after 2 days were weighed and placed in individually numbered cages without food or water. They were again weighed 2 days later, and placed in the room kept at 2-5°C. During this period the animals were observed and weighed at 2- to 5-day intervals and measurements on the rate of eruption were made after 6 to 16 days. After 12 to 59 days, the animals were returned to the laboratory (about 21°C), weighed and given food and water *ad libitum.* Two days later alizarine red S was injected for the second time, and 2 days post-injection the animals were sacrificed.

---

[1] Schour, I., and Steadman, S. R., *Anat. Rec.*, 1935, **63**, 325.

[2] Schour, I., Hoffman, M., Sarnat, B. G., and Engel, M. B., to be published.

* From the distance between the alizarine red S lines of the animals kept at 2-5°C, 76.8 u was subtracted to correct for the 4 days before and 2 days after when the animals were at room temperature. 6 × 12.8 u (average rate of dentin apposition per day in control animals) = 76.8 u.

Control Group (21°C, average room temperature). These animals were given standard rat ration. The rate of eruption was dedetermined during a 14-day period. Alizarine red S was given at the beginning and the end of a 21-day period. The animals were sacrificed 2 days later.

Findings. The results of these experiments are summarized in Tables I and II.

Discussion. The average rate of eruption in control animals at room temperature was 0.8 and 1.1 mm per week in the upper and lower incisors respectively (Table II). In Table I, with one exception, it will be noted that the rate of eruption was markedly retarded. This suppression was approximately proportional to the percent of time the animals hibernated. In the group which hibernated from 0-20% of the time the rate of eruption of the lower incisors was 2.2 mm per week (twice the control). As yet no adequate explanation can be offered for this.

The average rate of dentin apposition in the 9 control animals at room temperature was 12.8 u per day (Table II). This is in marked contrast to the rate of dentin apposition in the animals kept at 2-5°C (Table I). In the group of 7 animals which hibernated 76-100% the rate was 1.5 u per day or 12% of the control. It is of interest to note that 3 of these animals were at 2-5°C for 59 days, whereas the remaining 4 animals were kept for only 21 days.

TABLE I.
Average Rate of Eruption and Dentin Apposition of Incisor at 2-5°C.

% Hibernation (days)*	Rate of eruption (mm per week) Upper	Lower	Rate of dentin apposition (u per day)	Weight loss (g per day) 21°C†	2-5°C
76-100	0.1	0.1	1.5	10.0	1.4
21-75	0.2	1.0	2.5	9.1	2.7
0-20	0.4	2.2	6.6	8.5	5.8

*Approximate percentage of total period at 2-5°C in which animals were found in the state of hibernation.

†No food, no water, for 2 days.

TABLE II.
Average Rate of Eruption and Dentin Apposition of Incisor at 21°C. (Average Room Temperature).

Rate of Eruption (mm per week)	Upper	0.8
	Lower	1.1
Rate of dentin apposition (u per day)		12.8

The rate of apposition, however, was the same. In 6 animals which hibernated less (21-75%) the rate was increased to 2.5 u per day or 20% of the control. In 13 animals which hibernated between 0 and 20%, the rate was 6.6 u per day, or 52% of the control. This was the only group in which there were any mortalities; 11 of the 13 animals died. The foregoing demonstrates the marked suppression of the activity of the dentin-forming cells of animals kept at cold temperatures and hibernating for variable periods of time.

The daily weight loss was greatest in the animals which hibernated least. Conversely, those animals which hibernated most had the smallest weight loss (Table I). These findings are consistent with those of the rate of eruption and dentin apposition.

Summary. The rate of eruption and dentin apposition of the incisor in the thirteen-lined ground squirrel kept at 2-5°C is markedly retarded and, further, is retarded in approximate proportion to the number of days the animal hibernates.

13007 P

Renal Concentration Test Employing Use of Pituitary Extracts. Response of Normal Subjects.

W. A. SODEMAN AND H. T. ENGELHARDT.

From the Department of Medicine, School of Medicine, Tulane University, New Orleans, La.

The various concentration tests for renal function in use today consist primarily of some form of water restriction sufficient to reduce urinary volume. The specific gravity of urine is thereby elevated a variable degree depending upon tubular function and the degree of water restriction. We have attempted to shorten or abolish the long period of water deprivation necessary in concentration tests by the use of the antidiuretic action of posterior pituitary extracts. The effectiveness of posterior pituitary extracts in raising the specific gravity of the urine has recently been shown in dogs by Paine and Nelson.[1] Comparison of a standard concentration test for renal function with the use of posterior pituitary extracts was obtained by submitting patients to three or more of the following 5 procedures: (1) A modified Fishberg concentration test with restriction of fluids from 6 o'clock the previous evening. The overnight urine was discarded and half-hourly specimens collected from 9 o'clock in the morning until 11 or 12 noon; (2) the same procedure with the administration of 0.5 cc (10 units) of surgical Pituitrin subcutaneously at 9 o'clock in the morning; (3) free access to fluids until 9 in the morning when they were restricted; the Pituitrin administered and 30-minute specimens taken as above; (4) free access to fluids until 9 in the morning when, in 15 minutes, 1,600 cc of water were given followed by administration of 0.5 cc of surgical Pituitrin; specimens of urine were again collected at half-hourly intervals; (5) the fourth procedure was repeated without the administration of the Pituitrin. Specific gravities were obtained by weighing the urine in a 2 cc weighing bottle or pyknometer to 0.1 mg and comparison made with a similar quantity of distilled water at the same temperature according to the usual technic.[2]

---

[1] Paine, W. G., and Nelson, E. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 729.

Fifty-two experiments have been carried out on 15 normal individuals. Consistent results have been obtained in all. Fig. 1 shows the results in one subject. The curves are numbered to correspond to the procedures described above. In this instance, the test was

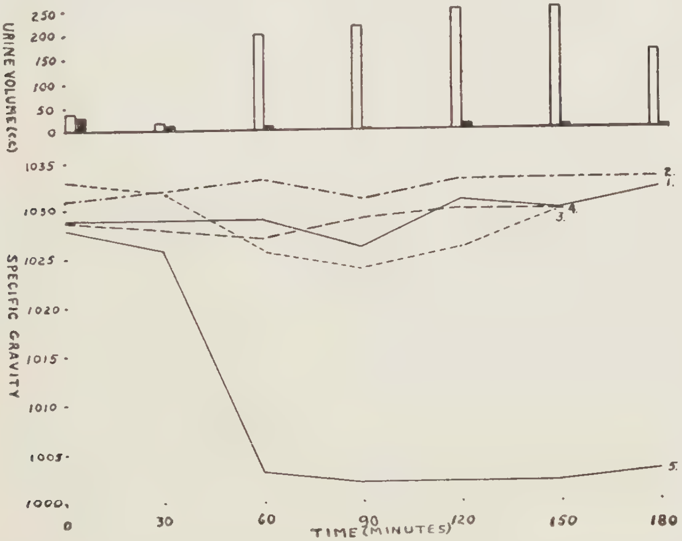


Fig. 1.

Results in one subject. The curves are numbered to correspond to the procedures described in the text. Black and white columns represent urinary volume corresponding to curves 4 (1600 cc water with Pituitrin) and 5 (1600 cc water without Pituitrin) respectively.

---

[2] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry,* Volume II, Williams and Wilkins Co., Baltimore, 1931.

carried out in very hot weather, which accounts for the fact that with or without preparation the urinary specific gravity started in each experiment in a range (above 1.025) indicative of satisfactory renal function. This fortunate circumstance revealed data which indicated that when water diuresis was inhibited by water restriction (Curve 2), or by excessive loss by perspiration or other routes of excretion (Curve 3), the addition of the pituitary extract had little or no effect in elevating the specific gravity. Since pituitary extract inhibits water diuresis, when the latter is already eliminated, little effect is to be expected upon the specific gravity. However, water diuresis instituted by the administration of 1,600 cc of water in 15 minutes at the beginning of the procedure (Curve 5) is inhibited by Pituitrin (Curve 4) indicating the effectiveness of this function of Pituitrin. This is also reflected in the urinary volumes which, with Pituitrin, were maintained at the initial low values (Fig. 1). Regardless of preparation of the patient, the administration of Pituitrin maintained the specific gravities at levels diagnostic of adequate tubular function.

Fig. 2 represents results obtained in a normal individual with (1) modified Fishberg test, (2) modified Fishberg test plus Pituitrin, and (3) no preparation plus Pituitrin. In this subject the initial

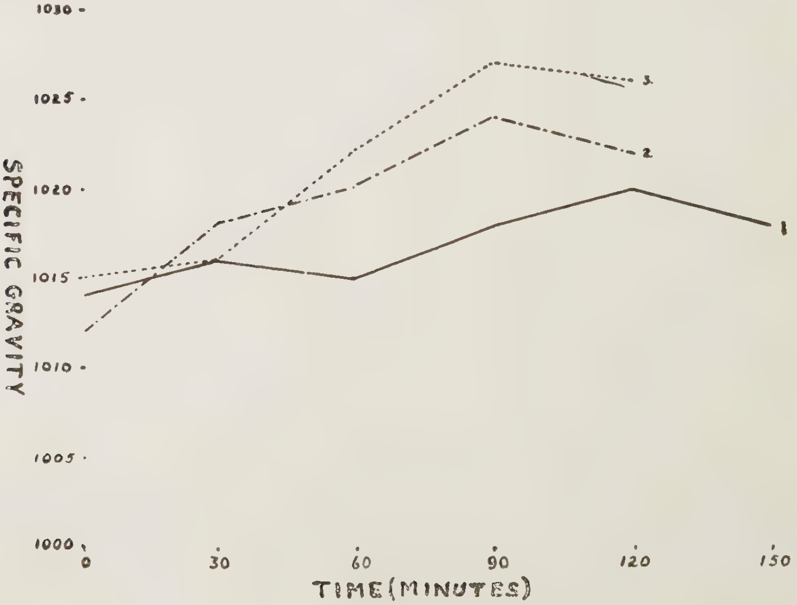


FIG. 2.

Results obtained in a subject with initial low urinary specific gravity. The curves are numbered to correspond to the procedures described in the text.

values of the specific gravity started in a low range not diagnostic of adequate renal function, and were elevated to diagnostic levels only when Pituitrin was added. It appears that without a period of water restriction, water diuresis was inhibited sufficiently by Pituitrin to give values considered indicative of adequate renal function. In all normal subjects studied, the results have been similar and in each instance specific gravity values were equal to or exceeded those obtained by the standard test with water restriction.

These results indicate that posterior pituitary extract in the doses used produced in individuals with normal renal function an inhibition of excretion of water sufficient to bring the specific gravity to or above the levels obtained with usual test periods of water restriction.

13008

Differentiation of Urogastrone and Pituitrin.*

John S. Gray, Stanley C. Harris and E. Wieczorowski.
(Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Urogastrone, a substance which inhibits gastric secretion and motility, has been obtained by a number of investigators from human urine.[1, 2, 3] The pressor principle of the posterior lobe of the pituitary, which also inhibits gastric secretion[4] and motility,[5] has likewise been obtained from human urine.[6, 7] Because of the similarities in the sources and properties of the two substances, it appeared desirable to investigate the possibility of their being iden-

---

* Aided in part by a grant from the Committee on Endocrinology of the National Research Council.

[1] Friedman, M. H. F., Recknagel, R. O., Sandweiss, D. J., and Patterson, T. L., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 509.

[2] Necheles, H., Hanke, M. E., and Fantl, E., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 618.

[3] Gray, J. S., Wieczorowski, E., Culmer, C. U., and Adkison, J. L., *Am. J. Physiol.*, 1940, **129**, 589.

[4] Cutting, W. C., Dodds, E. C., Noble, R. L., and Williams, P. C., *Proc. Roy. Soc. London, Ser. B*, 1937, **123**, 27.

[5] Quigley, J. P., and Barnes, B. O., *Am. J. Physiol.*, 1930, **95**, 7.

[6] Teel, H. M., and Reid, D. E., *Endocrinol.*, 1939, **24**, 297.

[7] Robinson, F. H., and Farr, L. E., *Ann. Int. Med.*, 1940, **14**, 42.

tical. Accordingly, a comparison has been made of the effects of urogastrone and pituitrin on gastric secretion, gastric motility, urine excretion, and blood pressure.

Methods. The effects on gastric secretion were measured by the double histamine injection technic,[3] using 6 dogs with pouches of the entire stomach. The effects on gastric motility were determined in 2 trained, unanesthetized dogs in which gastric motility was initiated and maintained by the introduction of approximately 80 cc of air into the recording balloon. The duration of inhibition, measured from the time when it appeared until the original tone and motility returned was taken as the indication of potency.[8] For the assay of anti-diuretic potency, diuresis was induced in a trained, unanesthetized dog with a bladder fistula by the administration of 250 cc of water by stomach tube. The ability of urogastrone and pituitrin to modify the resulting curve of diuresis was determined. For the assay of pressor or depressor effects dogs under ether anesthesia were used.

In all cases injections of the two principles were made intravenously. Pituitrin (Parke-Davis and Company) was used throughout. The urogastrone was a pyrogen-free product prepared by a method as yet unpublished.

Results. The results of the various assays are presented in the table and graph. It will be noted that as far as gastric secretory effects are concerned, 3 mg of urogastrone and 4 units of pituitrin exerted approximately equal degrees of inhibition. However, this

TABLE I.
Comparative Effects of Urogastrone and Pituitrin on Gastric Secretion and Motility and on Blood Pressure.

	Urogastrone				Pituitrin			
			Effect				Effect	
Assay	Dose, mg	No. assays	Degree	Kind	Dose, units	No. assays	Degree	Kind
Gastric Secretion	3	6	62%	Inhibition	.25	12	12%	Inhibition
	5	6	76 ″	″	1.0	6	29 ″	″
					2.0	6	37 ″	″
					4.0	5	53 ″	″
Gastric Motility	0.3	2	1.1 min	″	.001	2	None	″
	0.6	2	1.7 ″	″	.05	4	1.7 min	″
	2	3	4.0 ″	″	.1	4	2.9 ″	″
	3	3	4.6 ″	″	.25	2	13.8 ″	″
	5	2	5.8 ″	″	.5	2	18.2 ″	″
Blood Pressure	3	2	None		4	4	3.2 cm	Rise
	6	2	″					
	15	1	1 cm	Fall				

---

[8] Gray, J. S., Bradley, W. B., and Ivy, A. C., *Am. J. Physiol.*, 1939, **118**, 463.

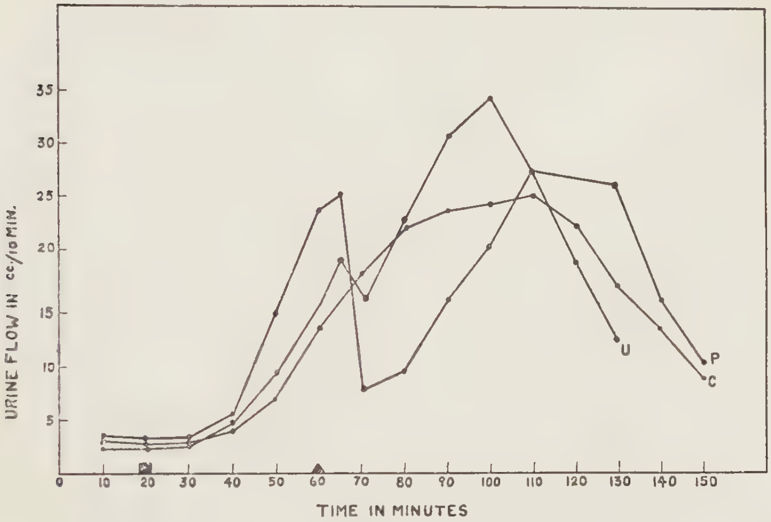


FIG. 1.

Antidiuretic effects of urogastrone and pituitrin. Water was given at ■ and drugs at ▲. Curve C is the average of 7 control experiments; curve U is the average of 5 urogastrone experiments (3 mg dose); curve P is the average of 3 pituitrin experiments (0.001 u. dose).

ratio holds for none of the other effects studied. It required only 0.1 unit of pituitrin to inhibit gastric motility as effectively as 1-2 mg of urogastrone. In regard to anti-diuretic action, 3 mg of urogastrone produced a barely detectable effect, whereas as little as 0.001 unit of pituitrin produced a striking effect. Finally, urogastrone has no effect on blood pressure unless very large doses are used, whereas 4 units of pituitrin produce an easily demonstrable pressor effect. There can be little doubt, therefore, that urogastrone and pituitrin are separate entities.

The effect of urogastrone on gastric motility is so mild as to render its significance questionable. The bearing of this finding on the hypothesis that urogastrone represents excreted enterogastrone requires further investigation.

Although pituitrin is a potent inhibitor of gastric secretion, the fact that enormous doses, in terms of antidiuretic effect, are required, argues against the possibility that the posterior lobe of the hypophysis exerts an important control over the gastric glands.

Conclusions. A comparison of the effects of urogastrone and pituitrin on gastric secretion, gastric motility, urine excretion, and blood pressure, indicates that the two principles are separate entities.

13009 P

Phosphorus Metabolism in Neoplastic Tissue.*

L. A. Erf† and J. H. Lawrence.
From the Crocker Radiation Laboratory and the Department of Medicine, University of California.

Recent studies[1, 2, 3] (with the aid of radioactive phosphorus) on normal mice, leukemic mice and on mice carrying various neoplasms, have shown that wherever there is leukemic or neoplastic infiltration there is a higher uptake of labelled phosphorus than in uninvolved tissues and this uptake is found to be higher than that of any other soft tissue. These facts together with the knowledge that bone and bone marrow take up more phosphorus than any other tissue have led to the use of radio-phosphorus in the treatment of leukemia.[4, 5, 6] The studies here reported concern the uptake of radio-phosphorus in the tissues of a small group of patients suffering from various neoplastic diseases. Most of these patients were in the terminal stages of their diseases and were given radioactive phosphorus in the form of sodium phosphate shortly before death.

Methods. The phosphorus was prepared by bombarding red phosphorus with deuterons in the cyclotron, thereafter was converted to a neutral solution of sodium phosphate (isotonic); and was administered orally. The pieces of tissues assayed were those obtained at the surgical and the postmortem tables. None had been embalmed, nor was the blood removed from them. After weighing, each sample was ashed at 450°C, and the radioactivity measured by means of a Lauritsen electroscope.

Results and Discussion. The assays are recorded in Table I. With one exception, the tissues were obtained between the fifth and ninth days following the last administration of radio-phosphorus. The low assay values of the tissues of the patient receiving radio-

---

* This work was aided by a grant from the National Advisory Cancer Council.

† William R. Kenan, Jr., Fellow.

[1] Jones, H. B., Chaikoff, I. L., and Lawrence, J. H., *Am. J. Cancer,* 1940, **40**, 235, 243.

[2] Lawrence, J. H., Tuttle, L. W., Scott, K. G., and Connor, C. L., *J. Clin. Invest.,* 1940, **19**, 267.

[3] Tuttle, L. W., Erf, L. A., and Lawrence, J. H., *J. Clin. Invest.,* 1941, **20**, 57.

[4] Tuttle, L. W., Scott, K. G., and Lawrence, J. H., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 20.

[5] Lawrence, J. H., Scott, K. G., and Tuttle, L. W., Studies on leukemia with the aid of radioactive phosphorus, *New International Clinics,* Vol. III, Series 2, Copyright, 1939.

[6] Lawrence, J. H., *Radiology,* 1940, **35**, 51.

TABLE I.
Radioactivity Assays of Autopsied (except Case 7) Tissues.
(Expressed in microcuries per gram wet weight.)

	Neuro-blastoma	Seminoma	Melanoma	Ewing's Sarcoma	Hodgkins		Lympho-sarcoma	Fibro-sarcoma
	Rich	*Gaa*	*Hun*	*L. Gre*	*Cla*	*Hor*	*Bea*	*A. Gre*
Oral admin. of P^{32} in Mc	5.3	6.0	7.0	1.68	4.0	1.7	5.1	7.1
Amt in g of Sod. Phos. in which P^{32} was inc.	2.8	1.05	1.5	.3	.5	.57	1.27	1.092
Days between ad. of P^{32} and Aut.	9	6	7	5	60	9	7	7
Tumor	.100	.138	1.480					.0108
Mesenteric lymph nodes	.093	.278*		.045 .038*	.006†	.024*	.020*	
Sternal marrow	.253				.016	.018		
Rib c marrow	.070	.070		.052		.015	.031	
Spleen	.084	.064	.232	.040	.012	.024	.047	
Liver	.118	.094	.391	.032	.012	.036	.050	
Stomach	.053	.032		.025				
Kidney	.087	.076	.283	.033	.010	.020	.035	
Lung	.072		.090	.030 .042*	.006	.031	.039	
Heart muscle	.072	.057	.173	.028	.011	.008	.025	
Pancreas	.097	.054	.350	.043		.025	.038	
Cerebrum			.074	.004				
Skin		.025		.011		.0007		
Surrounding muscle								.001

*Infiltrated.
†Fibrotic.

phosphorus 60 days before death are due to the excretion and the decay of radio-phosphorus (half-life of P^{32} is 14.3 days). In the patients with seminoma and melanoma the malignant tissues or tissues infiltrated with malignant cells retained much more P^{32} than the other tissues assayed. And, in case 7, the biopsied piece of fibrosarcoma retained more radio-phosphorus than the surrounding muscle. In those patients with neuroblastoma, Ewing's sarcoma, and one with actively progressing Hodgkin's disease, the abnormal tissues retained as much radio-phosphorus as did those more rapidly metabolizing organs such as liver and kidney. In the other case of Hodgkin's disease the nodes involved were densely fibrosed and contained less P^{32} than most of the other tissues assayed. The lymphosarcoma tissue of one patient contained less P^{32} than other tissues assayed, but transplantable lymphosarcomas of mice do not have this characteristic.[1]

Conclusions. Of the tissues assayed the majority of the malignant tissues or those infiltrated with malignant cells retain as much or more radio-phosphorus per gram wet weight than other rapidly metabolizing tissues of the same patients.

13010

Possibility of Producing Arterial Hypertension by Intracisternal Injection of Kaolin.*

Piero P. Foa', Carl F. List and Max M. Peet. (Introduced by L. H. Newburgh.)

From the Department of Neurosurgery, University of Michigan Medical School, Ann Arbor, Michigan.

Experimental hypertension by means of intracisternal injection of kaolin was produced in dogs first by Dixon and Heller,[1] and Heller.[2] These authors observed a considerable elevation of blood pressure lasting many months. According to Heller, this type of hypertension is "central" in nature and due to increased intracranial pressure resulting from mechanical obstruction of the subarachnoid and ventricular spaces. Braun,[3] and Braun and Samet[4, 5, 6] showed that hypertension produced by injection of kaolin can be prevented or abolished by denervation of the kidneys. Bilateral adrenalectomy, on the other hand, does not prevent the development of this form of hypertension (Jeffers, Lindauer and Lukens[7]). Hoff[8] obtained a sustained increase of blood pressure of 90 mm Hg in 4 dogs. Since he could reduce the blood pressure by ventricular puncture, Hoff assumed that the elevation of blood pressure resulted from stimulation of the basal ganglia and mammillary bodies by increased intracranial pressure. Further successful results with the kaolin method were reported by Giordano and Galigani.[9, 10] Griffith and associates produced hypertension in a large number of rats by the same procedure,[11–14] but the high blood pressure was maintained for more than 2 months in only a few animals.

---

* Funds for this work were available through a grant to the Department of Neurosurgery by the Aaron Mendelson Memorial Trust Fund.

[1] Dixon, W. E., and Heller, H., *Arch. exp. Path. u. Pharm.*, 1932, **166**, 265.

[2] Heller, H., *Wiener Klin. Wschr.*, 1934, **13**, 241.

[3] Braun, L., *Wiener Klin. Wschr.*, 1933, **46**, 225.

[4] Braun, L., and Samet, B., *Wiener Klin. Wschr.*, 1934, **47**, 65.

[5] Braun, L., and Samet, B., *Arch. exp. Path. u. Pharm.*, 1934, **177**, 662.

[6] Braun, L., and Samet, B., *Wiener Klin. Wschr.*, 1935, **48**, 940.

[7] Jeffers, W. A., Lindauer, M. A., and Lukens, F. D. W., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 260.

[8] Hoff, H., *Wiener Klin. Wschr.*, 1935, **48**, 545.

[9] Giordano, C., and Galigani, D., *Minerva Medica*, 1937, **2**, 395.

[10] Giordano, C., and Galigani, D., *Arch. It. di Med. Sperim.*, supple. No. 5, 1939.

[11] Griffith, J. Q., Jeffers, W. A., and Lindauer, M. A., *Am. J. Physiol.*, 1935, **113**, 285.

In contrast to these successful results we were unable to obtain any significant increase in blood pressure in dogs after intracisternal kaolin injections.

Fourteen dogs were used in our experiments. The blood pressure was taken 6 to 9 times over a period of at least one month prior to the injection of kaolin. During the recording, the dogs, which were unanesthetized and trained to the procedure, remained perfectly calm and coöperative. After the average normal blood pressure had been thus established, kaolin was injected and then the blood pressure recorded at frequent intervals for a period of 32-61 days. One dog received a second injection of kaolin after 61 days of observation and the blood pressure was frequently recorded during a period of 56 more days.

Blood pressure was recorded directly by puncture of the femoral artery with a 21 gauge needle connected with a mercury manometer, the tubing of which contained a 5% solution of sodium citrate as anticoagulant.

The animals were anesthetized by intravenous administration of evipal or nembutal. Twenty mg per kg of kaolin were suspended in 3-5 cc of sterile physiological saline solution and injected into the cerebello-medullary cistern after withdrawal of an equal amount of spinal fluid.[†] After the injection, the dogs were kept on the operating table for 20 minutes with their heads lowered to facilitate the passage of kaolin into the ventricles and basilar cisterns.

The average blood pressure of 14 healthy dogs was found to vary between 102 and 155 mm Hg (average 120), but was fairly constant for the individual animal. Five of the dogs died within a few days following the cisternal puncture.

Of the remaining 9 dogs, 4 showed a moderate increase of blood pressure (30, 32, 38, and 39 mm Hg respectively) lasting for a period no longer than 20 days. Then, the blood pressure receded to its previous normal level. The remaining 5 dogs exhibited an increase in blood pressure of only 6, 10, 15, 19, and 19 mm Hg respectively, which is well within the normal range of physiologic fluctuations or within the possible range of error.

Autopsies, performed in 4 dogs, revealed considerable internal hydrocephalus of the lateral, third and fourth ventricles. Most of

---

[12] Griffith, J. Q., and Roberts, E., *Am. J. Physiol.*, 1938, **124**, 86.

[13] Lindauer, M. A., Griffith, J. Q., and Jeffers, W. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 497.

[14] Freeman, N. E., and Jeffers, W. A., *Am. J. Phys.*, 1939-40, **128**, 662.

[†] In 6 dogs commercial kaolin USP was used; collosol kaolin was used in 8 dogs.

the kaolin injected was found in the basilar cisterns and subarachnoid spaces and only a minimal amount, if any, was noted in the fourth ventricle.

We are unable to explain why our results differ from those of other authors and why we failed to produce lasting arterial hypertension. We used the same kind and amount of kaolin as other investigators (no difference was found in the action of the two preparations of kaolin used); only our method of recording the blood pressure was different. Whereas, other authors used the indirect cuff method, we preferred the direct arterial puncture, the reliability of which has been proven by many recent investigators. We also checked the accuracy of our blood pressure readings by occasional use of larger cannulas (18 and 17 gauge).

Summary. A suspension of kaolin injected into the cerebellomedullary cistern of 9 dogs failed to produce a significant or sustained elevation of blood pressure, although considerable internal hydrocephalus was present in all autopsied dogs.

13011

Production of Deciduomata in Ovariectomized Mice Receiving Progesterone.*

CHARLES W. HOOKER. (Introduced by Edgar Allen.)

From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn.

It has been recognized since the original experiments of Loeb[1] that the corpus luteum hormone is essential for the development of the maternal portion of the placenta. The belief has been quite general,[2] however, that this hormone must be supplemented by estrogen for the reaction to occur. Apparently the only instance of the induction of placentomata with progesterone alone is reported by Selye,[3] who traumatized the uterus of an ovariectomized rat receiving 15 mg of progesterone daily. In the experiments reported here much smaller amounts of progesterone were effective in eliciting the decidual reaction in uteri of mice traumatized longer than 2 months after ovariectomy.

---

* These experiments were aided by a grant from the Fluid Research Fund of Yale University School of Medicine.

[1] Loeb, L., *J. A. M. A.*, 1908, **50**, 1897.

[2] Allen, E., Hisaw, F. L., and Gardner, W. U., Chapter VIII in *Sex and Internal Secretions,* edited by Allen, E., Danforth, C. H., and Doisy, E. A., 1939.

[3] Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 343.

The observations were made on 17 young adult, ovariectomized mice. Ten days after ovariectomy one horn of the uterus in 7 animals was slit throughout its length and 0.5 mg of progesterone[†] daily was injected subcutaneously beginning at operation. The 2 animals autopsied one day and one animal autopsied 2 days after operation showed developing deciduomata. One animal on the third day showed no decidual reaction. The remaining 3 animals had well developed deciduomata on the second, third, and fourth days.

In another group of 6 mice 68 days after ovariectomy one horn of the uterus was slit throughout its length and 3 sutures were placed in the other horn, and the animals were killed 6 days later. Daily injections of 0.5 mg of progesterone were started 7 days before operation in 4 animals and at operation in 2 animals and continued until autopsy. Six days after operation placentomata were found in 3 of the pretreated animals and in one of the 2 animals started on progesterone at operation.

In a final group of 4 mice 70 days after ovariectomy one horn of the uterus was slit and threads placed in the other horn as before. Progesterone was injected daily beginning 3 days before operation, the dose being 0.25 mg in 2 animals and 1.0 mg in 2 animals. At autopsy 3 days after operation deciduomata were present in 3 animals and absent in one of the animals receiving the lower dose.

In most instances the deciduomata were macroscopic, bead-like endometrial thickenings, composed in all cases of large, basophilic, often multinucleated cells, in which mitoses were numerous. In the smaller and younger deciduomata all intermediate stages of transformation of cells of the endometrial stroma into decidual cells could be seen. Apparently the uterine trauma merely exaggerated greatly the enlargement of the stroma cells which characteristically occurs in the mouse in response to progesterone.[4] Administration of the progesterone prior to traumatizing the uterus resulted in larger placentomata than treatment started at the time of operation.

Uterine trauma in 78 spayed mice receiving estrogen or androgen or no treatment consistently failed to result in decidual reactions.[5] Various amounts of progesterone over various periods have likewise consistently failed to elicit deciduomata in 70 ovariectomized mice not subjected to uterine traumatization.

[†] The progesterone was supplied by Ciba Pharmaceutical Products, Inc., through the courtesy of Mr. R. C. Mautner. This substance was dissolved in sesame oil in such concentrations that each of the doses was contained in 0.05 cc of oil.

[4] Hooker, C. W., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 270.

[5] Hooker, C. W., *Anat. Rec.*, 1941, **79**, 211.

The mouse is apparently capable of producing progesterone in amounts equivalent to 1.0 mg injected daily;[6] and the equivalent of the smaller injected amounts is assuredly produced normally by the ovaries of the mouse during pregnancy, pseudopregnancy, and lactation.[4, 6] Moreover, the long period after ovariectomy precludes any direct involvement of estrogen as such in these reactions, and presumably also rules out any preparatory action of this hormone.

Thus, without the recent or simultaneous action of estrogen, progesterone in physiologic amounts is capable of eliciting the development of placentomata in the suitably traumatized uterus of the mouse.

13012

Capsulation of *Neisseria gonorrhoeae*.

LEO H. T. BERNSTEIN. (Introduced by J. Howard Brown.)

From the Department of Pathology and Bacteriology, Johns Hopkins University, Baltimore, Maryland.

We have succeeded in demonstrating and staining the capsule of *Neisseria gonorrhoeae* by a simple method. The presence of a capsule about the gonococcus has been reported only a few times and not substantiated, so that the Committee for Survey of Research on the Gonococcus and Gonococcal Infections[1] concluded, "The opinion of the majority is that the gonococcus does not form a capsule." Israeli[2] using a modified Hiss capsule stain found a clear or lightly stained zone, one to 3 times as wide as the organism; Szelvasi[3] claimed that in older cultures the capsule stained more intensively, although he did not describe the capsule. Recently Almaden[4] mordanted smears by Bailey's method, staining with carbolfuchsin, and stated that capsules could be found when the organisms were in the mucoid state.

In our work various technics have been tried, and finally Churchman and Emelianoff's[5] method, with modifications, was successful.

---

[6] Pfeiffer, C. A., and Hooker, C. W., *Am. J. Physiol.*, 1940, **131**, 441.

[1] Bayne-Jones, S., Chairman, *Am. J. Syph., Gon. and Ven. Dis.*, 1936, **20**, 5.

[2] Israeli, C., *J. Am. Med. Assn.*, 1921, **76**, 1497.

[3] Szelvasi, J., *Dermatolische Wschr.*, 1932, **94**, 204.

[4] Almaden, P. J., *J. Infect. Dis.*, 1938, **62**, 36.

[5] Churchman, J. H., and Emelianoff, N. V., *J. Exp. Med.*, 1933, **57**, 485.

Absolutely clean slides as used for flagella stains were employed; a drop of a slightly turbid aqueous suspension of the organism was placed at one end of the slide, the slide tilted and the drop allowed to run down and air dry. Then 10 drops of Wright's stain was placed on the slide and, when almost evaporated, washed off with tap water, distilled water, or buffered solution (Clark and Lubs, pH 6.4). To demonstrate capsules by this method a metallic sheen must surface the slide and provide a smooth pink-purple background and not a granular one.

The organisms used were 2 strains of gonococci isolated 2 months previously by Dr. R. D. Reid from clinical cases of gonorrhea. Dr. Reid kindly furnished 24-48-hour cultures grown on either dextrose starch agar or ascitic infusion agar; in the latter case the organisms were washed several times with physiological salt solution to free them of whatever protein material might have come from the medium. These cultures were rechecked for identity: they were Gram negative cocci seen most frequently in the typical biscuit shaped diplococcal form, fermented dextrose and not maltose, formed whitish-grey, translucent, raised colonies with smooth outline, one millimeter in diameter on chocolate-blood infusion agar, and these colonies gave a strong oxidase reaction with the paradimethylanalinemonohydrochloride reagent.

By the method described, preparations were obtained as shown in photomicrographs 1-5. The capsule is seen as a clear or very light pink halo, having a violet-pink outer membrane, and surrounds a blue stained soma, all against a smooth homogeneous background. The capsule may surround single organisms, diplococci, tetrads or groups of 8, the typical cleavage patterns as described by Neisser.[6] The cocci are .8 by 1 mu and the capsule (about diplococci) 2 by 3 mu.

In order to make certain that the clear zone was a true structural capsule and not an artifact, efforts were made to stain it more differentially; this was accomplished by flooding the dried smear with 5% acetic acid for 2 minutes, washing with water, allowing to dry and then staining as before. Photomicrograph 6 was taken of such a preparation and the capsule was seen as a red structure with a dark red outer membrane, surrounding the blue somatic body. Due to the chemical treatment the capsule is slightly smaller than that seen in photomicrograph 5, from the same suspension, but stained without preliminary fixation.

Moist India ink observations were also made to compare find-

---

[6] Neisser, A., *Deutsche Med. Wschr.*, 1882, **8**, 1852.

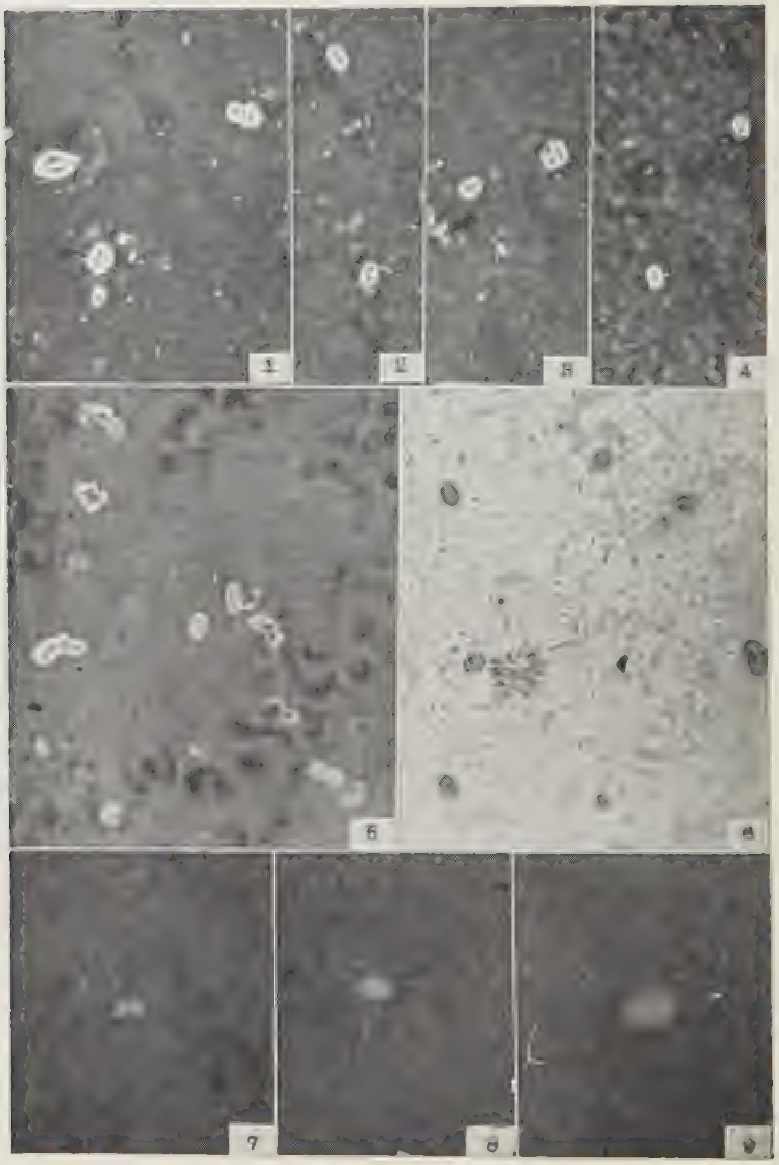


FIG. 1.

Photomicrographs 1000 ×.

1-5. *Neisseria gonorrhoeae,* Wright's stain.
6. *Neisseria gonorrhoeae,* fixed with acetic acid, then Wright's stain.
7-9. *Neisseria gonorrhoeae,* moist India ink preparations.

ings. The same appearances as in the stained slides were evident; photomicrographs 7-9 are of moist India ink preparations and show the light, more refractile capsule about the darker somatic body of the gonococcus. The capsule possessed structural rigidity and appeared to have a definite elastic outer membrane, as the India ink particles were seen to cause indentations when they hit up against it. The ink particles then rebounded, as though caused to do so by the contraction again of the membrane.

13013

Effects of Increased Metabolism on Ketosis

D. R. DRURY AND A. N. WICK.

From the Department of Physiology, School of Medicine, University of Southern California, Los Angeles, and the Scripps Metabolic Clinic, La Jolla, Calif.

In animals in a state of ketosis exercise has been reported not to change significantly the rate of excretion of ketone bodies.[1, 2] Increased activity of isolated diabetic muscles causes marked increase in rate of disappearance of these substances.[3] Elsewhere we have suggested that these apparently contradictory findings can be reconciled if the liver increases its production of ketone bodies as a result of exercise.[4] To test this hypothesis, the ketone excretion of the human subject on a constant ketogenic diet was determined during several days. The urine was divided into day and night portions at 8 A.M. and 8 P.M. In several different experiments each lasting several days, the amount of muscular activity during the daytime period was increased on certain days by strenuous exercise (tennis) for at least 2 hours and on some days it was cut down to a minimum by rest in bed. The ordinary activity was light laboratory work. Regular hours for sleep were maintained during the night periods throughout. From Table I it is obvious that except for days of complete inactivity the nocturnal excretion of ketone bodies is definitely higher than the diurnal. There is also a tendency for the daytime output to be less, the greater the activity. At the same time the night periods which follow days of high activity tend to have more ketonuria than those which follow days

---

[1] Barker, S. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 893; *J. Physiol.*, 1939-40, **97**, 394.

[2] Drury, D. R., *California and Western Medicine*, 1936, **45**, 1.

[3] Blixenkrone-Moller, N., *Hoppe-Seyler's Z. f. Physiol. Chemie.*, 1938, **253**, 423.

[4] Barnes, R. H., Drury, D. R., Greeley, P. O., and Wick, A. N., *Am. J. Physiol.*, 1940. **130**, 144.

TABLE I.

Ketone Body Excretion in Urine, as Grams per Period of Human on Constant Ketogenic Diet.

Day	Period	Exp. 1	Exp. 2	Exp. 3	Exp. 4
1	Day	E 1.68	.83	1.26	.69
	Night	8.25	2.26	2.24	1.11
2	Day	.95	.75	E 1.08	E 1.33
	Night	3.71	2.03	6.15	2.18
3	Day	E .68	E .67	E 2.51	R 2.96
	Night	5.42	4.30	5.88	1.73
4	Day	1.20	1.37	R 5.50	E .53
	Night	2.86	3.83	5.22	2.04
5	Day	R 2.59	E .85	2.91	
	Night	3.45	4.07	4.44	
6	Day		1.44		
	Night		3.54		

E—Indicates strenuous exercise (tennis 2 hours or more) at some time during the period.

R—Indicates rest in bed during the period.

The diet was constant during every experiment. There were slight differences in diet between the different experiments.

of low activity. These results would suggest that muscular activity decreases the concentration of ketone bodies in the body at the time of this activity but that in the subsequent rest period the rate of production of these substances by the liver is accelerated.

This question was investigated by a special experiment carried out on 2 successive days with the subject on the constant ketogenic diet. On the afternoon of the first day he engaged in heavy exercise (tennis) and kept at rest during the evening. On the following day he rested in the afternoon and engaged in ordinary activity during the evening. In another pair of days on the same constant diet, the subject engaged in ordinary activity on the first day, exercised in the afternoon of the second day and rested in the period after that. The results are given in Table II and again indicate that ketone excretion is not increased during exercise but may be augmented in a period of rest following the activity.

These results may be due to decreased function of the kidneys during exercise. It is known that the rate of urea excretion is diminished during strenuous exercise.[5] In our next experiments we followed the effect of exercise on the blood ketone body level, using the method of Barnes and Wick.[6] In preliminary experiments it

---

[5] Addis, T., and Drury, D. R., *J. Biol. Chem.*, 1923, **55**, 629.

[6] Barnes, R. N., and Wick, A. N., *J. Biol. Chem.*, 1939, **131**, 413.

TABLE II.
Urinary Excretion of Ketone Bodies in mg per Hour.

	Exp. 1			Exp. 2	
Time	1st day	2nd day	Time	1st day	2nd day
11 P.M.- 7 A.M.	150	126	12 M. - 6 A.M.	86	58
7 A.M.-10 ”	161	172	6 A.M.-12 N.	17	38
10 ” - 1 P.M.	58	77	12 N. - 6 P.M.	14	E 26
1 P.M.- 4 ”	E 18	R 108	6 P.M.-12 M.	24	R 217
4 ” - 7 ”	59	75			
7 ” -11 ”	R 109	82			

E—Indicates strenuous exercise during the period.
R—Indicates rest in bed during the period.

TABLE III.
Effect of Exercise on Blood Ketone Level. Values Expressed as mg Ketone Bodies per 100 cc Blood.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Immediately before exercise	7.7	15.7	11.9	6.6
Immediately after exercise	3.7	9.6	8.9	4.3

Exercise was running at medium steady rate for 20 minutes.

was found that a fast of 20 hours was adequate to produce a definite ketonemia with the blood ketone level steadily rising as the fast was continued. The method of procedure adopted was as follows: After a fast of 20 hours a blood sample for ketone body determination was taken. The subject then exercised for about 20 minutes. The exercise was running at a steady medium pace—not enough to give an oxygen debt. Another blood sample was taken on completion of the exercise. The results are given in Table III. There is a significant drop in all cases as a result of the exercise. In 5 control experiments it was found that the average rate of rise of the blood ketone level after 20 hours of fasting was 2.2 mg % per hour. The actual drop caused by the exercise is then greater than appears from the differences found. We can conclude that a short brisk period of exercise will give a definite lowering of the blood ketone concentration and hence that changes in ketone excretions in the urine caused by exercise are not due simply to change in function of the kidneys during this time.

Summary. The human subject in a ketogenic state excretes less ketone bodies during exercise than ordinarily. There seems to be an increased excretion in a subsequent rest period. The blood concentration of the ketone bodies is also decreased during a short period of exercise.

13014

Comparison of Macro- and Microgravimetric Technic for Lipid Analysis of Serum.*

Arild E. Hansen.

From the Department of Pediatrics, University of Minnesota, Minneapolis.

Wilson and Hansen[1] using a microgravimetric technic for the determination of lipids found the average molecular weight of the serum fatty acids to be higher than that usually assumed. Later, it was observed that the fatty acids in the phospholipid (acetone insoluble) fraction had even higher average molecular weights.[2] In an attempt to determine whether these high values might be due to the small amount of material employed, a study was carried out wherein the results obtained by the use of this microgravimetric technic could be compared with those obtained by the use of similar procedures but with larger quantities of material. In addition, comparison was made of the iodine numbers when both small and large quantities of lipid material were used.

Blood was obtained from a 4-year-old child with a known lipemia (nephrosis). The lipids were extracted from 20 cc of serum with 400 cc of the usual Bloor mixture (alcohol-ether—3:1). After evaporation to dryness, the lipids were reëxtracted with petroleum ether and brought to a volume of 250 cc. Aliquots of 5 cc (containing 0.4 cc serum) were used for the microdetermination;[1] 100 cc aliquots (containing 8.0 cc serum) were used for the macro, the same steps being followed as in the micro method. The iodine absorption was estimated by Yasuda's micro technic[3] for the small sample and by the Hanus method for the large sample. The results obtained are presented in Table I.

Rather remarkable is the fact that the results obtained by both methods for the amount of saponifiable material recovered and for the molecular weights agree so closely. As seen in Table I the average of the total fatty acids recovered by the micro method was 1666 and by the macro method 1660 mg per 100 cc serum. The titration values for the serum fatty acids, and consequently the calculated values for the average molecular weights, were likewise

---

* Aided by grants from Mead Johnson and Company and the Medical Graduate Research Fund of the University of Minnesota.

[1] Wilson, W. R., and Hansen, Arild E., *J. Biol. Chem.*, 1935, **112**, 457.

[2] Hansen, Arild E., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 376.

[3] Yasuda, M., *J. Biol. Chem.*, 1931-2, **94**, 401.

TABLE I.
Values of Total Fatty Acids (Saponifiable Fraction) of Serum Lipids as Obtained by Macro- and Microgravimetric Technics.

	Micro method	Macro method
Aliquots	0.40 cc serum	8.0 cc serum
Wt. of Sap. Fraction	6.64 mg	132.9 mg
	6.69	132.7
	6.66	—
	1659.3 mg/100 cc	1661.2 mg/100 cc
	1673.6	1658.8
	1664.0	—
Titration Value	2.28 cc .01N NaOH	4.55 cc .1N NaOH
	2.28	4.55
	2.28	—
M.W. (calc.)	291.4	292.1
	293.4	291.7
	292.1	—
I.N.	99.5*	104.9†
	99.8	105.6
	101.1	—

*Yasuda technic.
†Hanus method.

practically uniform by both methods. As regards the iodine numbers of the fatty acids, we found that the smaller quantity gave lower values than the larger quantity, the average iodine number being 100.1 by the Yasuda micro method and 105.2 by the Hanus method.

Because the iodine values obtained by the use of the pyridine sulfate dibromide reagent, which is employed in the Yasuda method, tend to be lower than those obtained by certain other methods and lower than those which would be theoretically expected,[3] the question arose as to whether more accurate results might be obtained by dilution of the Hanus reagent for use with micro quantities of fat. For this reason, determinations of iodine numbers of known solutions (mixtures of palmitic acid and linolic acid)[†] were made using: Yasuda's technic, the Hanus method, and the Hanus method with reagents diluted for adaptation to a micro technic. Representative data from the 10 experiments performed are shown in Table II. These reveal that the iodine numbers obtained by the usual Hanus method are practically identical with the theoretical numbers for these known substances. They also show that the modification of

---

[†] The linolic acid was obtained through the courtesy of Dr. J. P. Kass of the Department of Botany of the University of Minnesota. This fatty acid was chemically pure and was found to have an iodine number of 180 by the Wijs method and 178 by the Hanus method.

TABLE II.

Iodine Numbers of Mixtures of Palmitic Acid and Linolic Acid as Determined by the Hanus Method, Hanus Method Adapted to Micro Proportions, and the Yasuda Technic.

Mixture No.	Hanus	Micro Hanus	Yasuda	Theoretical
A-1	87.7 87.7 87.6	82.3 81.0 —	79.1 79.7 79.7	88.7
A-2	115.6 115.2 115.3	105.3 104.6 105.6	105.3 105.5 105.5	115.6
B-1	89.0 89.3 —	83.5 83.8 —	83.2 81.9 83.4	88.9
B-2	117.7 117.3 —	108.8 107.3 —	107.0 106.8 106.3	115.6

this technic to a micro method results in values very similar to those obtained by the use of the Yasuda method. Both the micro methods, however, give results from 6 to 10 points lower than those obtained by the macro method and those which would theoretically be expected.

Summary and Conclusions. Studies of the serum fatty acids from a child with a known lipemia (nephrosis) by means of the microgravimetric technic of Wilson and Hansen were made and the values thus obtained compared with those found by using 20 times the amount of fatty material. The results showed the amounts of saponifiable fraction recovered, the titration values, and the calculated molecular weights to be almost identical by both the micro and macro methods. Those obtained for the iodine numbers, however, by the use of the Yasuda micro method were consistently somewhat lower than those obtained by the use of the Hanus method and lower than would theoretically be expected. Dilution of the reagents used in the Hanus method for adaptation to a micro technic, resulted in iodine number values practically identical with those obtained by the Yasuda micro method.

From the data presented it seems warranted to conclude that the microgravimetric is a satisfactory technic for lipid analyses. Although the degree of unsaturation of the lipids tends to be somewhat lower when micro quantities are used, the pyridine sulfate dibromide reagent gives results comparable to the Hanus reagent diluted for adaptation to micro quantities.

13015

Partial Purification of the Virus of Epidemic Influenza by Adsorption on Calcium Phosphate.*

JONAS E. SALK. (Introduced by Thomas Francis, Jr.)

From the Department of Bacteriology, New York University College of Medicine.

The purpose of the present communication is to summarize certain of a series of experiments on the partial purification of influenza virus using calcium phosphate as the adsorbing agent. The procedure is simple and the amount of material that can be handled is limited only by the volume of infected lung suspension or chick embryonic tissue suspension available.

The procedure herein employed is a modification of a previously reported method[1, †] for the removal of bacteria from large quantities of broth culture. By the addition of calcium chloride and phosphate buffer, at pH 7.5, a precipitate of calcium phosphate is formed in the broth culture. The precipitate adsorbs the bacteria, settles quickly, and can readily be separated from the supernatant fluid. The bacteria are then "eluted" from the calcium phosphate by adjusting the hydrogen ion concentration of the resuspended precipitate to about pH 4.5. This causes the calcium phosphate to go into solution, leaving the bacteria suspended in a small volume of fluid from which they can be separated by centrifugation. Because of the instability of epidemic influenza virus when exposed to an acidity as high as pH 4.5,[2] this method for the removal of calcium phosphate could not be employed. Accordingly, the modification introduced was one in which the calcium phosphate was dissolved at neutrality by the use of the sodium salt of citric acid.

In this report, a typical experiment will be described in which a comparison is made between the infectivity, the immunogenicity, and the nitrogen content in the untreated and the partially purified preparation of a suspension of infected mouse lung. The amount of residual infective and immunogenic material which remained in the supernatant fluid after adsorption was also tested.

---

* This study was conducted under a grant from the International Health Division of the Rockefeller Foundation.

[1] Salk, J. E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 228.

[†] Calcium phosphate has been employed for the purification of the diphtheria toxin (Roux, E., and Yersin, A., *Ann. Inst. Past.*, 1889, **3**, 273; Abt, G., *Ann. Inst. Past.*, 1928, **42**, 1336); and for the purification of the type specific polysaccharide of the pneumococcus (Felton, L. D., Kauffman, G., and Stahl, H. J., *J. Bact.*, 1935, **29**, 149).

[2] Stock, C. C., and Francis, T., Jr., *J. Exp. Med.*, 1940, **71**, 661.

2.8 grams of the lung tissue of mice infected with the PR-8 strain of influenza virus were ground with alundum and diluted with 11.2 cc of physiological salt solution and 16.8 cc of 0.05 M phosphate buffer (pH 7.8) to form a 10% suspension. This was centrifuged for 25 minutes in the Swedish Angle Centrifuge to remove gross particles of tissue. One portion was partially purified in the following manner involving 3 successive adsorptions, although it has since been found that only 2 are necessary. To 15 cc of the suspension in a centrifuge tube, 4.5 cc of 0.1 M calcium chloride and 4.9 cc of 0.1 M sodium hydroxide were added. The optimal hydrogen ion concentration for the precipitation and adsorption was found to be in the range of pH 8.0 to 8.4 as determined with a glass electrode. The reagents were introduced drop by drop, with constant mixing, in order that the precipitate might form evenly throughout the suspension. When the reagents were added in this manner, the precipitate that formed was very flocculent, settled quickly, leaving a crystal clear supernatant fluid. After spinning in the ordinary centrifuge, the separated supernatant fluid was again treated with 4.5 cc of 0.1 M phosphate buffer at pH 7.8, 4.5 cc of 0.1 M calcium chloride and 4.9 cc of 0.1 M sodium hydroxide, and centrifuged. To the second supernatant fluid the same volumes of phosphate buffer and calcium chloride and 4.5 cc of the sodium hydroxide were added. After the third centrifugation, the resultant supernatant fluid, which is ordinarily discarded, was set aside to be tested for residual infective and immunizing potency. The pH of 9.39 of the final supernatant fluid was corrected to pH 7.16 by the addition of 3.5 cc of 0.1 M hydrochloric acid. The precipitates of calcium phosphate were almost pure white and the supernatant fluid was bright red, indicating that little or none of the hemoglobin was adsorbed.

The 3 precipitates were pooled together and washed twice, with 30 and 20 cc, respectively, of 0.05 M phosphate buffer (pH 7.8). The washed precipitate was suspended in 2.5 cc of 1.0 M sodium citrate and 12 cc of 0.1 M hydrochloric acid. (In subsequent experiments citric acid has been used instead of hydrochloric acid.) The pH of the resulting mixture was approximately 6.5. A small amount of precipitate which remained undissolved was separated and treated with 0.7 cc of 1.0 M sodium citrate and 3.0 cc of 0.1 M hydrochloric acid. A small residue, insoluble in sodium citrate, was discarded and the 2 opalescent supernatant fluids were pooled. The final pH of 6.18 was changed to 6.61 by the addition of 4.0 cc of 0.1 M sodium hydroxide. This citrate solution was dialyzed in

TABLE I.
Comparison of Activity and Nitrogen Content of the Untreated and the Partially Purified Preparations.

	Dilutions	Untreated suspension	Partially purified fraction	Supernatant fluid
Titer of Infectivity	10^{-2}	4,4,++	4,4,4	8,++++,++
	10^{-3}			++,++,+
	10^{-4}	5,6,6	5,5,6	
	10^{-5}	6,6,6	++++,+++,++	
	10^{-6}	+++,++,+	++,++,+	
Nitrogen Content[3]	mg per cc 10% susp.	1.46	0.132	
	mg per M.I.D.*	7.3×10^{-7}	6.6×10^{-8}	

Numerals denote days of death of individual mice after inoculation with 0.05 cc of suspension, intranasally, while in ether anesthesia.

Survivors were autopsied on the 10th day after infection.

+ to ++++ represent increasingly greater degrees of pulmonary involvement in the survivors.

*M.I.D. refers to Minimal Infective Dose.

the ice-box for 1½ hours, against 3 changes of 10 volumes of distilled water. The dialyzed solution was then used as the partially purified virus preparation and tested for infectivity, immunogenic potency and nitrogen content. The crude and partially purified preparations and the adsorbed supernatant fluid were all diluted to comparable volumes before use. The results are shown in Table I.

It is clear from these data that the amount of virus activity remaining in the supernatant fluid after adsorption is very little when compared with the original. In fact it appears to be not more than 0.1 of 1% of the amount present in the crude suspension. However, the infective titer of the partially purified fraction compares quite favorably with the titer of the untreated suspension. Although there is a difference in the potency of the 10^{-5} dilutions in this experiment, this is not very great when it is considered that the course of a fatal infection in 2 of the 3 mice, in the group tested with the partially purified fraction, was interrupted by a termination of the experiment. The close similarity in infective titer of these 2 preparations, in view of the residual activity of the discarded supernatant fluid, suggests that little, if any, loss in virus activity occurs in the process of washing, elution, and dialysis. On the other hand, the nitrogen content of the partially purified fraction is distinctly less than that of the untreated, being about 9% of the original. Thus it has been possible to eliminate more than 90% of the nitrogenous impurities with a loss of very little of the virus activity. From a comparison of the nitrogen content of the minimal infective dose of the original and the partially purified preparations it might be said that a purification of approximately 11 times has been effected. Preliminary tests for stability of the partially purified material indicate that the infectious titer is essentially unchanged after standing at ice-box temperature for 24 hours. Efforts toward further purification are in progress. One procedure being tested involves alternate rapid freezing and thawing of the citrate solution. By this means the finely suspended material coalesces and settles out as a precipitate. Approximately one-half of the remaining nitrogen-containing material can be removed in this fashion. However, the amount of reduction in activity which may result has not yet been determined.

---

[3] Levy, M., and Palmer, A. H., *J. Biol. Chem.*, 1940, **136**, 57.

Mice were immunized intraperitoneally with the crude material, the partially purified material, and the discarded supernatant fluid. A quantitative estimate of the immunogenicity of these preparations, made in the manner described by Francis,[4] revealed that the immunizing potency for mice paralleled the infective potency.

A comparison was made of the amount of nitrogenous material separated from both normal and infected lungs by the calcium phosphate method. No significant differences were observed. It seems, therefore, that the virus of epidemic influenza can be adsorbed by calcium phosphate in a manner similar to a fraction of normal tissue. This observation is in keeping with those reported by Kabat and Furth,[5] who studied the virus of leukosis and of sarcoma of fowls, using the ultracentrifuge; by Claude,[6] studying the agent causing mouse sarcoma and chicken tumor I; and by Taylor, Sharp, Finkelstein, and Beard,[7] working with chick embryonic tissue diseased with the virus of equine encephalomyelitis.

The degree of purification achieved in the present experiments, in which a chemical method was used, is of the same order as that obtained with the ultracentrifuge in investigations on certain other viruses.[5, 6, 8] More detailed studies are in progress and will be reported subsequently.

---

[4] Francis, T., Jr., *J. Exp. Med.*, 1939, **69**, 283.

[5] Kabat, E. A., and Furth, J., *J. Exp. Med.*, 1940, **71**, 55.

[6] Claude, A., *Science*, 1940, **91**, 77.

[7] Taylor, A. R., Sharp, D. G., Finkelstein, H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 462.

[8] Hughes, T. P., Pickels, E. G., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1938, **67**, 941.

13016

The Serological Activity of Extra-embryonic Fluids of Chick Infected with Virus of Influenza A.

Werner Henle and Leslie A. Chambers.* (Introduced by D. W. Bronk.)

From the Department of Pediatrics, Medical School, University of Pennsylvania, and the Children's Hospital of Philadelphia.

The use of the fertile hen's egg for the propagation and study of the virus of influenza A has been limited to the chorio-allantoic membrane, the embryo and the yolk sac. Nothing was known of the virus content of the allantoic and amniotic fluids until the recent publication of Burnet[1] whose findings agree with the observations made in this laboratory during the past several months.

Several mouse-adapted strains of influenza A virus have been transferred to chick embryos by injecting 0.5 ml amounts of mouse lung filtrates or pooled extra-embryonic fluids through the air space into the allantoic sac of fertile eggs which previously had been incubated for 9 to 10 days. Accidental injection into the amnion, embryo or yolk sac was unlikely because of the mobility of these structures. After further incubation for 24 to 48 hours the extra-embryonic fluids (amniotic and allantoic) were usually harvested together and were studied separately in only a few experiments. From 3 to 8 ml of fluid were obtained from the allantoic sac and 1.0 to 2.5 ml from the amnion. The amniotic fluid was frequently quite clear while the allantoic fluids showed various degrees of turbidity. On standing in the refrigerator, or on freezing and thawing, a precipitate was formed in many of the allantoic fluids, which was removed by centrifugation without apparent loss of serological activity.

The extra-embryonic fluids were both very rich in virus. For example, the WS-strain,[2] now in its 48th consecutive egg passage not only retained its virulence for mice but actually became lethal in much higher dilutions in these fluids than the original mouse lung material. Preparations were occasionally encountered of which as little as 0.05 ml of a 10^{-10} dilution proved fatal for white mice. The intraäbdominal injection of such fluids produced immunity in mice against the mouse-adapted influenza strains, and the antiinfluenzal mouse lung serum neutralized the egg-adapted virus. The F-12[3] and Fla-12[4] strains, in the 25th consecutive egg passage, behaved in a similar way. Other strains have been started too recently to allow inclusion in this report.

---

* Fellow of the Nemours Foundation.

[1] Burnet, F. M., *Austral. J. Exp. Biol. and Med. Sc.*, 1940, **18**, 353.

[2] Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2**, 66.

Since these virus preparations are obtained without grinding of tissues they seem to be particularly suited for studies in the purification of influenza virus as the interference of normal cellular components[5] is reduced considerably. The low content of non-viral proteins (total protein 0.03 to 0.05%) in this fluid in connection with its high virus titer suggests its suitability as a vaccine. When produced under conditions defined below the fluids, and the allantoic fluid in particular, contain a satisfactory titer of complement fixation antigen and show a flocculation phenomenon when mixed with convalescent or immune sera.

The application of the complement fixation reaction to influenza A is well established.[6] For this test dilutions of the pooled extraembryonic fluids, serum and complement were mixed in equal volumes (0.2 ml) and incubated for one hour at 37°C. After the addition of 0.4 ml of sensitized sheep cells (2.5%) the test was further incubated for 60 minutes when final readings were made. Two units each of amboceptor and complement were employed. Anticomplementary reactions, so frequent with mouse lung antigens have not been observed with these fluids. Normal egg fluid never gave complement fixation in the presence of convalescent or immune sera.

In contrast with the complement fixation reaction little is known about aggregation phenomena with preparations containing influenza A virus. Magill and Francis[7] observed a flocculation of crude influenzal mouse lung suspensions by human convalescent sera. Our attempts to obtain a specific agglutination of influenza A virus preparations, derived from infected mouse lungs by high speed centrifugation, were unsuccessful. Whether this failure was due to insufficient amounts of virus[8] or to a mechanical hindrance through normal lung particles[5] present in these suspensions is unknown. Mouse lung preparations with as much as 10^8 to 10^{10} infectious units per ml for white mice did not clump in the presence of immune sera against tissue culture virus, or of convalescent sera. However, when the pooled extra-embryonic fluids, clarified by horizontal centrifugation, were mixed with equal amounts of human or ferret convalescent sera (0.2 ml each), a flocculation was observed after incubation for one hour at 37° C, overnight refrigeration, and centrifugation for 10 minutes at 1500 r.p.m. The test was read after gentle tapping of the tubes. The floccules rising from the bottom tended to redisperse when shaken too vigorously.

---

[3] Stokes, J., Jr., and Wolman, I. J., *New International Clinics*, 1940, **1**, 115.

[4] Magill, T. P., and Francis, T., Jr., *Brit. J. Exp. Path.*, 1938, **19**, 273.

[5] Henle, W., and Chambers, L. A., *Science*, 1940, **92**, 313.

[6] Nigg, C., Crowley, L. K., and Wilson, D. E., *Science*, 1940, **91**, 603. (Earlier references given here).

[7] Magill, T. P., and Francis, T., Jr., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 81.

[8] Merril, M. H., *J. Immunol.*, 1936, **30**, 169.

The complement fixation and flocculation antigens appear in the fluids simultaneously, but decidedly later than the infectivity. Table I shows that the pooled extra-embryonic fluids contained a high titer of virus after 12 hours while the complement fixation test was still negative. After 24 hours this reaction became slightly positive and reached its peak in 36 hours. Also the infectivity was highest at this time but decreased thereafter, while no such decrease was noted in the serological reactions and the flocculation, following otherwise closely the complement fixation reaction, was even more pronounced after 48 hours.

In order to obtain, therefore, sufficient complement fixation antigen, the allantoic sac should be inoculated with a dose of virus sufficiently dilute to allow the embryos to survive at least for 36 hours. The time of survival naturally depends on the dose of virus used as inoculum. Table II shows an experiment in which groups of 8 eggs were infected with different amounts of virus, and the fluids were harvested and pooled when 30 to 60% of the embryos had died. Thus the fluids of eggs inoculated with a more concentrated preparation (undiluted or 10^{-2}) had to be harvested after 24 hours, while the fluids of those injected with higher dilutions

TABLE I.

Test	Dilution of fluid	WS-42 (extra-embryonic fluids) harvested after				
		6 hr	12 hr	24 hr	36 hr	48 hr
Mouse Titration	10^{-5}	3 3 3	D6 D7 D10	D6 D6 D8	D5 D5 D6	D6 D7 D10
	10^{-6}	0 0 0	D8 D9 D10	D6 D11 3	D7 D8 D8	D7 D8 4
	10^{-7}	0 0 0	3 3 3	3 1 0	D10 D10 3	3 2 2
	10^{-8}		2 1 0	1 0 0	D8 D10 2	D4* 1 1
	10^{-9}		1 0 0	0 0 0	2 2 0	0 0 0
Complement Fixation	Undil.	0	0	++++	++++	++++
	½	0	0	+++	++++	++++
	¼	0	0	0	++++	++++
	⅛	0	0	0	+++	+++
	1/16	0	0	0	0	0
Flocculation	Undil.	0	0	±	+	++

*Eaten, not autopsied.

TABLE II.

Test	Dilution of fluid	WS-43 (extra-embryonic fluids) inoculated in dilution of					Normal extra-embryonic fluid
		Undiluted	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
Time of harvest		24 hr	24 hr	36 hr	36 hr	44 hr	40 hr
Mouse Titration	10^{-7}	2 2 2	D6 D7 0	D6 D6 D9	D6 D7 D7	D6 D8 D9	
	10^{-8}	1 1 0	D8 D10 3	D8 D9 0	D7 D10 D11	D9 D10 D11	
	10^{-9}	0 0 0	D10 D11 3	D8 D9 3	D8 D8 0	D7 D8 D8	
	10^{-10}	0 0 0	4 3 3	D9 D11 3	D8 0 0	3 2 2	
Complement Fixation	½	++++	++++	++++	++++	++++	0
	¼	+++	++++	++++	++++	++++	
	⅛	0	0	++++	++++	++++	
	1/16	0	0	++++	++++	+	
	1/32	0	0	+++	++++	0	
	0	0	0	0	0	0	
Flocculation	Undil.	±	±	++	++	+	0

were collected correspondingly later. In all these fluids the infectivity for mice was practically identical, regardless of the concentration of the inoculum, excepting the preparation derived from eggs inoculated with the undiluted virus suspension. The low infectivity of this fluid, confirmed by similar experiments with the F-12 strain, has not yet been explained. The complement fixation antigen was present in high titer only in fluids collected from eggs injected with the 10^{-3} or 10^{-4} dilution which were harvested after 36 hours. Inoculation of the eggs with fluid in a dilution of 10^{-5} led to approximately the same virus titer, but less complement fixation antigen was found in the harvested fluid. The flocculation test corresponded closely to the complement fixation reaction.

It was found that under the conditions outlined above the allantoic fluid regularly contained complement fixation and flocculation antigen while the amniotic fluid frequently was negative and if positive, showed always a weaker reaction than the allantoic fluid. This fluid, on the other hand, in the presence of convalescent serum, frequently gave a complete fixation of complement in a dilution of 1:32. It can be heated to 56°C for 20 minutes, or dried from the frozen state and redissolved in distilled water, without serious loss of activity.

The pooled extra-embryonic fluids were used as antigen for the study of human sera obtained during a recent epidemic of influenza A in a closed institution. Some of the results are recorded in Table III, which shows a comparison between the flocculation, complement fixing and neutralizing antibodies of the sera of several patients taken (a) during the acute stage, and (b) 3 weeks after the

TABLE III.

Patient	Serum	Flocculation with extra-embryonic fluid		Titer of compl. fixing antibodies	Titer of neutralizing antibodies	Virus isolated
		Influenzal	Normal			
B.S.	Acute	0	0	0	1:4	+
	Conval.	+	0	1:32	1:64	
F.B.	Acute	0	0	0	1:2	
	Conval.	+	0	1:32	1:128	n.t.
T.M.	Acute	0	0	1:2	n.t.	n.t.
	Conval.	+	0	1:16		
N.N.	Acute	0	0	0	n.t.	n.t.
	Conval.	+	0	1:16		
R.O.	Acute	0	0	0	n.t.	0
	Conval.	+	0	1:8		
K.T.	Acute	0	0	1:2	n.t.	0
	Conval.	±	0	1:8		
M.P.	Acute	0	0	1:2	1:16	0
	Conval.	±	0	1:4	1:64	
F.G.	Acute	0	0	1:2	1:4	n.t.
	Conval.	0	0	1:16	1:64	
M.M.	Acute	0	0	1:4	1:16	0
	Conval.	0	0	1:4	1:8	

n.t.—not tested.

onset of the disease. It is obvious from the table, that in all cases of influenza A showing a distinct rise in complement fixing and neutralizing antibodies, or from which the virus had been isolated (altogether 18 cases were observed) the flocculation was positive with one exception (F.G.). The flocculation seems to be correlated with the amount of complement fixing antibodies in that only sera with a titer of more than 1:8 showed a distinct flocculation while below this titer doubtful reactions, or none at all, were observed. One patient (M.M.) apparently did not suffer from influenza A since no rise in antibodies occurred, and correspondigly no flocculation was noticed.

Summary. The pooled extra-embryonic fluids of the chick infected with influenza A are very rich in virus. They can be used as an antigen for complement fixation when harvested at least 36 hours after inoculation of influenza A virus, the time for optimal yield depending on the dose of virus used as an inoculum. Furthermore, they show a flocculation phenomenon when mixed with convalescent or suitable immune sera. The allantoic fluid is a better antigen for the serological reactions than is the amniotic fluid.

AUTHORS' INDEX

(The numerals indicate the page.)

Abramson, D. I., and Fierst, S. M. Blood flow, Graves' disease, 39.
Abramson, H. A., Moore, D. H., and Gettner, H. H. Ragweed protein, 153.
Adams, J. M. Virus pneumonitis in newborn, 114.
Alexander, F. A. D. 553.
Allen, A. 572.
Allen, E. 511.
Allen, F. W. 436.
Almquist, H. J., and Mecchi, E. Gizzard erosions, bile acids, vit. K, cincophen, 168.
Althausen, T. L. 387.
Alvarez-Tostado, C. 151.
Anderson, E., and Joseph, M. Adrenalectomy, urine N and survival, 321.
Anderson, H. H. 26; 658; and P'An, S. Y. Anticonvulsants, 611.
Anderson, T. F. 272.
Ansbacher, S. Vit. K deficiency, 421.
Auer, J., and Krueger, H. Anaphylaxis, edema, 74; Histamine, edema, 75.
Axelrod, A. E., Spies, T. D., and Elvehjem, C. A. Riboflavin, tissue, 146.

Baeza-Herrerá, H. 76.
Baeza-Rosales, H. 76.
Baldwin, F. M., and Goldin, H. S. Testosterone, anal fin transformation, 283.
Baldwin, M. 446.
Ball, J. Estrogen, mating behavior, hypophysectomy, 669.
Bang, B. 527.
Bang, F. B., and Bang, B. Sulfonamides, gonococci, 527.
Barnes, L. L., Sperling, G., and Maynard, L. A. Bone development, Mn., 562.
Barnes, R. H. 355.
Bartley, S. H. 557.
Bass, C. C. Enteritis, ulcerative, cause, 250.
Beard, D. 419.
Beard, J. W. 419.
Beattie, M. W., Berlow, A., and Leshne, F. F. Serum reactions, test, 453.
Becker, E. R. Coccidium infection, vit. B_1, B_6, 494.
Beckwith, J. R., and Chanutin, A. Hypertension, blood vol., 66.
Bellolio, P. 164.
Ben Dor, B. Chick, K need, 341.
Bengtson, I. A. "Q" fever, complement fixation, 665.
Benham, R. W. Pityrosporum ovale, characteristics, 176.
Bergman, A. J. 670.
Berliner, R. W. 549.
Berlow, A. 453.
Bernheim, F., and Handler, P. Alcohols, substituted, oxidation, 470.
Bernstein, A. 582.
Bernstein, H. T. *Neisseria gonorrhoeae*, capsulation, 700.
Bing, R. J., and Zucker, M. B. Kidney, pressor amines, 343.
Bishop, G. H., and Bartley, S. H. Optic system activity 557; and O'Leary, J. Colliculus, superior, potentials, 680.
Biskind, G. R. Testosterone, inactivation, 452.
Blount, R. F. Vasopressin, diencephalon, 629.
Bodansky, O. 248.
Booth, M., Erythrocyte fragility, 640.
Borson, H. J. 387.
Boyd, E. M. Pentothal, thioethamyl, depressants, 98.
Boyd, W. L. 16.
Bremer, F. Neuronal synchronization, 627.
Brice, A. T. 596.
Briggs, A. P. Nicotinic acid excretion, pellagra, 374.
Britton, S. W. 678.
Brooks, M. M. Blood O_2 consumption, CO, methylene blue, 219.
Broun, G. O., Muether, R. O., Mezera, R. A., and Le Gier, M. Encephalitis, transmission, 601.
Brown, D. V. 478.
Bruger, M. 560.
Brunschwig, R. 298.
Butsch, W. L. 484.
Butts, J. S., Sinnhuber, R. O., and Dunn, M. S. Liver glycogen, tyrosine, 671.

Cameron, J. A. Blood CO reduction, X-ray, 558.
Carmichael, E. B., and Thompson, W. D. Delvinal sodium, 233.
Catchpole, H. R. 580.
Chambers, L. A. 713.
Chanutin, A. 66.
Charipper, H. A. 184, 409.
Chase, M. W. 223.
Chaume, J. 164.
Chen, K. C. Relapsing fever spirochete growth, 638.
Chen, Y. P., and Anderson, H. H. Relapsing fever, therapy, 658.
Cheng, T. Y. 603.
Chow, B. F. 644.
Christensen, L. R. Streptococcus fibrinolysin detn., 674.
Chu, H. I. 603; Liu, S. H., and Yu, T. F. Diabetes insipidus, electrolytes, 682.
Churney, L. Egg, fertilized, shearing force, 393.
Clark, W. G. Adrenalectomized guinea pig, maintenance, 253.
Clifton, C. E. 443.
Climenko, D. R., McChesney, E. W., and Messer, F. Sulfonamide, renal function, 124.
Cohen, E. J. 330.
Conant, N. F., and Howell, A., Jr. Blastomycosis, etiology, 426.
Corcoran, A. C., Kohlstaedt, K. G., and Page, I. H. Angiotonin, blood pressure, 244.
Corey, E. L., and Britton, S. W. Diabetes insipidus, hypophysis, 678.
Cortell, R. 157.
Cotler, H. Y., Kirchner, M. T., and Romano, M. Pneumococci, sulfapyridine fast, 241.
Co Tui. 136.
Coulston, F. 523.
Counts, E. 523.
Cournand, A., and Ranges, H. A. Heart, R. auricle catheterized, 462.

Dann, F. P. 507.
Day, A. A. 569.
Deakins, M. Tissues, calcified, composition, 62.
Deatherage, F. E., McConnell, K. P., and Mattill, H. A. Reproduction, pro-oxidants, 399.
De Beer, E. J., Drektar, L., and Flusser, B. Vit. K, administration, 535.
De Falco, R. J. Antigens, assay, 500.
DeGraff, A. C., and Lehman, R. A. Scillaridin, theophyllinated, toxicity, 36.
Deutsch, E. 424.
Dewey, V. C. Tetrahymena geleii, nutrition, 482.

Dicken, D. M. 449.
Dingle, J. H., and Seidman, L. R. Meningitis, influenza, therapy, 34, 131.
Dorfman, R. I. Isoandrosterone from testosterone, 351.
Doty, J. R. Kidney tubules, reabsorption amino acids, 129.
Doubilet, H. 136.
Dragstedt, C. A. 303, 576.
Drektar, L. 535.
Drury, D. R., and Wick, A. N. Ketosis, high metabolism, 703.
Dunn, M. S. 671.

Ebert, R. V., and Stead, E. A., Jr. Plasma vol., detn., 139.
Eckardt, R. E., Gyorgy, P., and Johnson, L. V. Bacterial growth, riboflavin synergist, 405.
Egana, E. 161.
Ehrlich, S. B. 560.
Eiler, J. J., and Allen, F. W. Ribonuclease, 436.
Elder, J. H. Sodium pregnandiol glucuroniadate, chimpanzee urine, 57.
Elvehjem, C. A. 100, 146.
Emerson, G. A., and Evans, H. M. Pantothenic acid, growth, graying, 655.
Engelhardt, H. T. 688.
Erf, L. A. Iron, radio, secretion in milk, 284; 428; and Lawrence, J. H. Cancer, P metabolism, 694.
Evans, H. M. 411; 655; Simpson, M. E., and Lyons, W. R. Lactogenic, placentoma, 586.
Evans, T. C., Robbie, W. A., Goodrich, J. P., and Slaughter, J. C. Radiosensitivity, new-born, low temp., 662.

Falk, K. G. 248.
Fazekas, J. F. 553.
Feldman, J. 572; Cortell, R., and Gellhorn, E. Cocaine, bulbocapnine, sympathetic and parasympathetic systems, 157.
Ferguson, J. H. Prothrombin stability, 80.
Ferrebee, J. W., Leigh, O. C., and Berliner, R. W. Lymph, dye passage from blood, 549.
Fierst, S. M. 39.
Figge, F. H. J. Tyrosinase, glutathione, dopa reaction, 269.
Fine, J. 487.
Finland, M. 131.
Fisher, R. S., and Peabody, W. A. Liver extr., Fe, 207.
Fleischmann, W. Neuromuscular disease, α-tocopherol, testosterone, creatine excretion, 94.
Flusser, B. 535.
Foa', P. P., List, C. F., and Peet, M. M. Hypertension production, kaolin, 696.
Foglia, V. G. Hearts, lymph, function, 598.
Frank, R. T. 1.
Freedman, A. M. 385.
Freedman, S. I. 187.
Friedman, R., and Master, A. M. Heart sounds, infarction, 574.
Friedman, S. M. Progesterone anesthesia, 197.
Frost, D. V., Moore, R. C., and Dann, F. P. Achromotrichia, pantothenic acid, 507.

Gardner, W. U., Allen, E., and Smith, G. M. Bile duct hyperplasia, estrogen, 511.
Gassner, F. X. 290.
Gellhorn, E. 64, 157; Allen, A., and Feldman, J. Blood insulin, psychosis, 572.
Gengerelli, J. A., and Holter, N. J. Nerve stimulation, electrical fields, 532.
Gettner, H. H., 153.
Glaubach, S. Morphine, codeine, synergism by sulfapyridine, 53.
Goldblatt, H., 492.
Goldfarb, W., and Wortis, J. Pyruvate, brain oxidations, 121.
Goldin, H. S. 283.
Goodrich, J. P. 662.
Gordon, A. S. 184, 409.
Graessle, O. 364.
Gray, J. S., Harris, S. C., and Wieczorowski, E. Urogastrone, pituitrin, 691.
Greengard, H., and Stein, I. F., Jr. Secretin, assay, 149.
Greep, R. O., Van Dyke, H. B., and Chow, B. F. Metakentrin, assay, 644.
Griffith, J. Q., Jr., 311.
Gyorgy, P. 405; and Goldblatt, H. Liver injury, dietary, 492.

Haig, C. 180.
Hamm, P., Harvey, R. B., and Miller, E. S. Stem "tracheal" fluid, minerals, 347.
Handler, P. 470.
Hansen, A. E. 325; Serum lipid detn., 706.
Harris, M. M., and Harris, R. S. Serum choline esterase, curare, β-erythroidin, 619; Serum choline esterase, curare, metrazol, 623.
Harris, R. S. 619, 623.
Harris, S. C. 691.
Harrison, S. P., and Ivy, A. C. Angiotonin, gall bladder, duodenum, 112.
Harrow, B. 582.
Harvey, R. B. 347.
Haury, V. G., Procaine convulsions, Hg, Pb, 309.
Hays, E. E. 194.
Hechter, O., Levine, R., and Soskin, S. Insulin, glycogenesis, sugar conc., 390.
Heckel, N. J., and Steinmetz, C. K. α-Estradiol benzoate, azoospermia, 174.
Heilman, D., and Herrell, W. E. Gramicidin, hemolytic effect, 182.
Heinbecker, P. 44.
Hemingway, A. 313.
Henle, W., and Chambers, L. A. Embryonic fluid, serology, influenza, 713.
Herrell, W. E. 182.
Herriott, R. M. Biuret, Folin's reagent, Cu, 642.
Himwich, H. E., Fazekas, J. F., and Alexander, F. A. D. Cyanide, iodoacetate, survival, 553.
Hines, H. M. 257.
Hirschmann, H. Isoandrosterone in virilism urine, 51.
Hoeppli, R. *Taenia Taeniafornis* infection, splenectomy, 29.
Hoff, H. E. 395.
Hogan, A. G., Richardson, L. R., Long, B., and Itschner, K. I. Vitamins needed by rat, 530.
Hollander, F., Klempner, E., and Frank, R. T. Androgen, assay, 1.
Holmes, C. A., Ojers, G., and Dragstedt, C. A. Shock, liver histamine, 576.
Holter, N. J. 532.
Honold, E. 9.
Hook, W. E. 685.
Hooker, C. W. Progesterone, deciduomata, 698.
Howell, A., Jr. 426.
Howitt, B. Encephalomyelitis, equine, rabies, 69.
Howitt, B. F., and VanHerick, W. Virus, poliomyelitis, adaptation, 431.
Hsu, H. C. 603.
Hu, C. H. 606.
Hubbard, R. S., and Butsch, W. L. Sulfonamides in bile, 484.
Hughes, R. 192.
Hunter, F. E. Sphingomyelin, radio P, 281.

Itschner, K. I. 530.
Ivy, A. C. 112; 569.

Johnson, C. A. 104.
Johnson, J. B. Hemophilia, oxalic acid, 496.
Johnson, L. V. 405.
Jones, H. P. 300.
Joseph, M. 321.
Jukes, T. H. Perosis, choline, gelatin, creatine, 155.
Jung, R. W. 569.

Kabat, H., and Freedman, A. M. Shock, epinephrine, 385.
Kahn, M. C. Tuberculosis, M. histiocytes, 630; Histiocytes, mobility, 635.
Kaltreider, N. L. 266.
Kapnick, I., and Fine, J. Serum complement, cortin, 487.
Kark, R., White, F. W., Souter, A. W., and Deutsch, E. Blood prothrombin, liver disease, 424.
Kaufman, W. 395.
Keeley, J. L. Sulfonamide, intraperitoneal, 458.
Kessler, M., and Gellhorn, E. Convulsions, insulin, adrenalin, 64.
Kety, S. S., and Letonoff, T. V. Lead poisoning, Na citrate treatment, 476.
King, F. H., and Leslie, A. Pneumonia, jaundice, blood O_2, 554.
Kirschbaum, A., and Pfeiffer, C. A. Melanin deposition, testosterone, 649.
Kirchner, M. T. 241.
Kissin, B., and Locks, M. O. Urolithiasis, urine citrate, 216.
Kleinman, A. 472.
Klempner, E. 1.
Klinefelter, H. F. Sulfonamides, *E. coli*, 591.
Kohlstaedt, K. G. 244.
Kohman, T. P., and Rusch, H. P. Nucleoprotein, tumor, metabolic activity, 403.
Kolin, A. Blood flow meter, 235.
Kropp, B., and Baldwin, M. *B. welchii*, nerve 446.
Krueger, A. P., and Pucheu, H. Phage production, thermal inhibition, 210.
Krueger, H. 74, 75.
Kuntz, A., and Van Buskirk, C. Bile flow inhibition, celiac plexus, 519.
Kurotchkin, T. J., and Wyckoff, R. W. G. Rickettsia vaccine, 223.
Kurtz, A. C. $^{\alpha}$-amino acids—color reaction, 339.
Kurzrok, R. 262.

La Due, J. S. Heart, lanatoside C, 651.
Landsteiner, K., and Chase, M. W. Quinine hypersensitivity, 223.
Landy, M., and Dicken, D. M. Biotin, synthesis, 449; and Wyeno, J. Sulfonamides, p-aminobenzoic acid, 59.
Last, J. H., and Hays, E. E. Liver extract, embryo blood picture, 194.
Lawrence, J. H. 694.
Lecannelier, S. 161.
Le Gier, M. 601.
Lehman, R. A. 36.
Leigh, O. C. 549.
Leshne, F. F. 453.
Leslie, A. 554.
Letonoff, T. V. 476.
Levenstein, I. 409; Gordon, A. S., and Charipper, H. A. Endocrine organs, *in vitro* maintenance, 184.
Levine, R. 390.
Levine, V. E. 192.
Lewis, A. A. 440.
Lewis, J. M., Bodansky, O., Falk, K. G., and McGuire, G. Blood vit. A, 248.
Li, R. C., Sah, P. P. T., and Anderson, H. H. Triacetin, toxicity, 26.
Liebow, A. A. 18.
Lightbody, H. D., Witt, E., and Kleinman, A. Arginase, liver, thyroxine, 472.
Lipschutz, A., Bellolio, P., Chaume, J., and Vargas, L. Estrogens, tumorigenic action, 164; Egana, E., Szabo, I., and Lecannelier, S. Estrogens, tumoral specificity, 161; Vargas, L., Baeza-Rosales, H., and Baeza-Herrera, H. Estrogen, tumorigenic, 76.
Lipton, M. A. 100.
List, C. F. 696.
Liu, S. H. 682; Chu, H. I., Yu, T. F., Hsu, H. C., and Cheng, T. Y. Vit. C deficiency, anemia, 603.
Locke, A., Locke, R. B., and Schlesinger, H. Sulfonamide tolerance, 79.
Locke, R. B. 79.
Locks, M. O. 216.
Long, B. 530.
Longacre, A. B., and Honold, E. Sulfonamides, gas gangrene, 9.
Loving, W. L. *B. leprae*, expl. infection, 293.
Luck, J. M., Nimmo, C., and Alvarez-Tostado, C. Albumin, liver, cleavage, 151.
Luzzatti, L., and Hansen, A. E. Sclerema neonatorum, serum lipids, 325.
Lyons, W. R. 586.

MacNeal, W. J. 545.
McCarty, M. Sulfonamides, p-aminobenzoic acid, 133.
McChesney, E. W. 124.
McConnell, K. P. 399.
McCravey, A. 513.
McGuire, G. 248.
McKee, C. M. 300.
McKenzie, F. F. 455.
McNutt, S. H. 547; and Trussell, R. E. *Trichomonas foetus* and *vaginalis*, growth, 489.
Machella, T. E., and Maguire, E. F. Liver cirrhosis, Vit. B, 502.
Macht, D. I., and Spencer, E. C. Muscle extr., toxicology, 228.
Mackay, E. M., and Barnes, R. H. Egg white disease, vit. B_6 and fatty acids cure, 355.
Magill, T. P. Influenza, repeated, 316; and Tyndall, M. Influenza virus, 371.
Maguire, E. F. 502.
Mannering, G. J., Lipton, M. A., and Elvehjem, C. A. Riboflavin need, fat intake, 100.
Manwell, R. D., Counts, E., and Coulston, F. Sulfonamides, avian malaria, 523.
Marshak, A., and Erf, L. A. Lymphoma implants, nuclei injection, 428.
Marx, W. 411.
Master, A. M. 574.
Mattill, H. A. 399.
Maynard, L. A. 562.
Mecchi, E. 168.
Meites, J., Bergman, A. J., and Turner, C. W. Lactogen, A. P., litter size, 670.
Mellish, C. H. 239.
Member, S., Ehrlich, S. B., and Bruger, M. Blood cholesterol distribution, 560.
Meneely, G. R., and Kaltreider, N. L. Pulmonary capacity, detn., 266.
Messer, F. 124.
Metcoff, J. 306.
Mezera, R. A. 601.
Miller, E. S. 347.
Mixner, J. P. 440; and Turner, C. W. Mamma, growth, involution, 437.
Moetsch, J. C. 361.
Monke, J. V. Gum acacia, non-available foodstuff, 178.
Moore, D. H. 153, 357.
Moore, R. C. 507.
Morse, L. M., and Schmidt, C. L. A. Vit. K absorption, bile fistula, 415.
Morton, H. E., and Anderson, T. F. *Coryne-*

bacterium diphtheriae, reduction of potassium tellurite, 272.
Muether, R. O. 601.

Nahum, L. H., Hoff, H. E., and Kaufman, W. Heart electrogram, temperature, 395.
Nielsen, K. 541.
Nier, A. O. 313.
Nimmo, C. 151.
Nungester, W. J. 187.
Nyboer, J., Spence, M. J., and MacNeal, W. J. Endocarditis lenta, heart sounds, 545.

Ojers, G. 576.
Okey, R. Cholesterol fed rats, 466.
O'Leary, J. 680.
Oppenheimer, M. J., and McCravey, A. Circulation time, pulmonary, 513.
Orr, W. F., and Moore, D. H. Prothrombin, 357.
Orten, J. M. 287.
Ouyang, G. 615.

Pader, M. 582.
Page, I. H. 244.
Pai, H. C., and Hu, C. H. *Leishmania donovani*, growth, 606.
Paley, A., and Seifter, J. Hydrofluoric acid burn, treatment, 190.
P'an, S. I. Sulfonamides in tissues, 31.
P'An, S. Y. 611.
Paschkis, K. E. Desoxycorticosterone, androgen, 336.
Patch, E. M. Cataracts, exp'l, 205.
Patek, A. J., Jr., and Haig, C. Dark adaptation, thyroid, α-dinitrophenol, 180.
Peabody, W. A. 207.
Pecher, C. Radio Ca, Sr, 86; and Pecher, J. Radio Ca, Sr, metabolism, 91.
Pecher, J. 91.
Peet, M. M. 696.
Perloff, W. H., and Kurzrok, R. Tumor, uterine, production by estrogen, 262.
Petersen, W. E. 379; Spielman, A., Pomeroy, B. S., and Boyd, W. L. Sexual behavior, thyroidectomy, 16.
Pfeiffer, C. A. 649.
Pickett, M. J., and Clifton, C. E. Glucose utilization, yeast, 443.
Pomeroy, B. S. 16.
Pucheu, H. 210.

Raiziss, G. W., Severac, M., and Moetsch, J. C. Sulfonamides, pneumonia, 361.
Rake, G., Shaffer, M. F., Jones, H. P., and McKee, C. M. Lymphogranuloma venereum, antigen, 300.
Ranges, H. A. 462.
Rasmussen, R. A., and Brunschwig, R. Cancer, gastric, pepsin, 298.
Reece, R. P. Androsterone, pituitary, mamma, 265.
Reinecke, R. M. 379.
Reinhardt, W. O., Marx, W., and Evans, H. M. Thymectomy, pituitary growth hormone, 411.
Richardson, L. R. 530.
Robbie, W. A. 662.
Rocha e Silva, M., and Dragstedt, C. A. Capillary permeability factor, 303.
Rolf, D. 44.
Romano, M. 241.
Rosenow, G. Leucocytosis, production, 5.
Rosenthal, L. Bacilli sporogenic, 448.
Rusch, H. P. 403.

Sachs, A., Levine, V. E., Schmit, A., and Hughes, R. Serum Cu, 192.
Sah, P. P. T. 26.
Salk, J. E. Virus, influenza, purification, 709.
Samuels, L. T., Reinecke, R. M., and Petersen, W. E. Mammary growth, nutrition, estradiol, 379.
Sarnat, B. G., and Hook, W. E. Tooth eruption, hibernation, 685.
Sayers, G., and Orten, J. M. Diabetes, NaCl, glucose disposition, 287.
Schattenberg, H. J., and Brown, D. V. *B. violaceus*, expl. infection, 478.
Schiller, A. A., and Struck, H. C. Bone, tensile strength, 198.
Schlesinger, H. 79.
Schmidt, C. L. A. 415.
Schmit, A. 192.
Schultz, E. W. Olfactory cells degeneration, 41.
Scudi, J. V., and Graessle, O. Streptococcus, p-caproylaminobenzenesulfonhydroxamide, sulfanilamide, 364.
Seidman, L. R. 34.
Seifter, J. 190.
Selye, H. Steroid hormones, anesthesia, 116; Testosterone, anesthesia, 142; and Nielsen, K. Uremia, desoxycorticosterone, blood N P N, 541.
Severac, M. 361.
Shaffner, M. F. 300.
Shafiroff, B. G. P., Co Tui, and Doubilet, H. Prothrombin time, Na citrate, 136.
Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W. Papilloma virus protein, 419.
Shorr, E., and Cohen, E. J. Vaginal epithelium, hormone effects, colchicine, 330.
Shuler, R. H. Gonads, ant. pituitary implants, 537.
Silver, S. Blood iodine, 213.
Simpson, M. E. 586.
Sinnhuber, R. O. 671.
Sklow, J. 276.
Slaughter, J. C. 662.
Smith, G. M. 511.
Snow, J. E., and Hines, H. M. Polyarthritis, jaundice, 257.
Sodeman, W. A., and Engelhardt, H. T. Kidney conc. test, pituitary extr., 688.
Somkin, E. Renin, cardiac output, 200.
Soskin, S. 390.
Souter, A. W. 424.
Spence, M. J. 545.
Spencer, E. C. 228.
Sperling, G. 562.
Spielman, A. 16.
Spies, T. D. 146.
Spirtes, M. A. Brain O_2 consumption, hyperthyroidization, 279.
Stapp, P. Skin, frog, electrical energy production, 382.
Starr, P., and Metcoff, J. Thyrotropic thyroid response, 306.
Stead, E. A., Jr. 139.
Stein, I. F., Jr. 149.
Stein, L., and Wertheimer, E. Adrenalectomy, intestinal absorption, 172.
Steinmetz, C. R. 174.
Stern, K. G. 18.
Stockholm, M., Althausen, T. L., and Borson, H. J. Thiamin, absorption, 387.
Stoesser, A. V. Sulfonamides, plasma lipids, 83.
Strauss, E., Dingle, J. H., and Finland, M. Sulfonamides, inhibition by p-aminobenzoic acid, 131.
Struck, H. C. 198.
Szabo, I. 161.

Takacs, W. S. 22, 319.
Taylor, A. R. 419.
Tennant, R., Liebow, A. A., and Stern, K. G. Embryonic material growth, 18.

Thomas, J. E. Cannula, gastric, intestinal fistulas, 260.
Thompson, W. D. 233.
Toomey, J. A., and Takacs, W. S. Virus, poliomyelitis, 22, 319.
Torstveit, O., and Mellish, C. H. Reflex, copulatory, adrenal cortical extr., 239.
Trentin, J. J., Mixner, J. P., Lewis, A. A., and Turner, C. W. Progesterone, assay, 440.
Trussell, R. E. 489; and McNutt, S. H. Gonococci, non-pathogenic, 547.
Tureen, L. L. Reflex, afferent stimulation, 543.
Turner, A. W. Milk, soft curd, 593.
Turner, C. W. 437, 440, 670.
Tyndall, M. 371.

VanBuskirk, C. 519.
Van Dyke, H. B. 644.
Van Herick, W. 431.
Van Wagenen, G., and Catchpole, H. R. Ovary, hysterectomy, 580.
Van Winkle, W., Jr. "Hypotensive" extr., 220.
Varco, R. L., and Visscher, M. B. Gastric elimination of dyes, 295.
Vargas, L. 76, 164.
Visscher, M. B. 295.
Vollmer, E. P., Gordon, A. S., Levenstein, I., and Charipper, H. A. Blood erythrocyte counts, hormones, 409.
Von Brand, T. Fat metabolism, 417.

Wagreich, H., Bernstein, A., Pader, M., and Harrow, B. Borneol, glucuronic acid, 582.
Wakerlin, G. E., and Johnson, C. A. Renin, B. P. reduction, 104.
Weir, J. M. Tuberculin antibodies, 47.
Weiss, P. Nerve centers, 14.
Werch, S. C., Day, A. A., Jung, R. W., and Ivy, A. C. Pectin, intestinal decomposition, 569.
Werkman, C. H. 313.
Wertheimer, E. 172.
West, R. Nicotinic acid, sulfapyridine antagonist, 369.
Weymouth, F. W., and Brice, A. T. Thyroid disease, BMR, respiration, 596.
White, F. W. 424.
White, H. L., Heinbecker, P., and Rolf, D. Kidney function, hypophysectomy, 44.
Wick, A. N. 703.
Wieczorowski, E. 691.
Wilgus, H. S., Jr., and Gassner, F. X. Reproduction, soybean meal, 290.
Winchester, C. F., and McKenzie, F. F. Semen, metabolic rate, 455.
Witt, E. 472.
Wong, S. C., and Ouyang, G. Tuberculin, assay, 615.
Wood, H. G., Werkman, C. H., Hemingway, A., and Nier, A. O. Propionic acid, bacterial synthesis, 313.
Woolley, D. W. Pantothenic acid, inositol, alopecia, 565.
Wortis, J. 121.
Wyckoff, R. W. G. 223.
Wyeno, J. 59.

Yen, C. H. Encephalitis virus, 609.
Youngner, J. S., Freedman, S. I., and Nungester, W. J. Shock prevention, histaminase, 187.
Yu, T. F. 603, 682.

Zinn, C. J., and Griffith, J. Q., Jr. Bone blood supply, sympathectomy, 311.
Zondek, B., and Sklow, J. Estrone inactivation by liver, 276.
Zucker, M. B. 343.

SUBJECT INDEX

(The numerals indicate the page.)

Achromotrichia, pantothenic acid, 507.
Adrenalectomy, guinea pig, maintenance, 253.
  intestinal absorption, 172.
  urine N, survival, 321.
Albumin, liver, cleavage, 151.
Alcohols, substituted, oxidation, 470.
α-amino acids—color reaction, 339.
Anaphylaxis, edema, 74.
Angiotonin, blood pressure, 244.
  gall bladder, duodenum, 112.
Anticonvulsants. 611.
Antigens, assay, 500.
Arginase, liver, thyroxine, 472.

B. leprae, expl. infection, 293.
B. violaceus, expl. infection, 478.
B. welchii, nerve, 446.
Bacteria, growth, riboflavin synergist, 405.
  sporogenic, 448.
Bile duct hyperplasia, estrogen, 511.
  flow inhibition, celiac plexus, 519.
Biuret, Folin's reagent, Cu., 642.
Blastomycosis, etiology, 426.
Blood cholesterol distribution, 560.
  CO reduction, X-ray, 558.
  erythrocyte counts, hormones, 409.
  flow, Graves' disease, 39.
  meter, 235.
  insulin, psychosis, 572.
  iodine, 213.
  O_2 consumption, CO, methylene blue, 219.
  Plasma vol., detn, 139.
  prothrombin, liver disease, 424.
  Serum choline esterase, curare, β-erythroidin, 619.
    curare, metrazol, 623.
    complement, cortin, 487.
    Cu, 192.
    lipid detn., 706.
    reactions, test, 453.
  vit. A, 248.
Bone blood supply, sympathectomy, 311.
  development, Mn, 562.
  tensile strength, 198.
Borneol, glucuronic acid, 582.
Brain O_2 consumption, hyperthyroidization, 279.

Ca, radio, 86.
  metabolism, 91.
Cancer, gastric, pepsin, 298.
  P metabolism, 694.
Cannula, gastric, intestinal fistulas, 260.
Capillary permeability factor, 303.
Cataracts, expl, 205.
Chick, K need, 341.
Cholesterol fed rats, 466.
Circulation time, pulmonary, 513.
Cocaine, bulbocapnine, sympathetic and parasympathetic systems, 157.
Coccidium infection, Vit. B_1, B_6, 494.
Colliculus, superior, potentials, 680.
Convulsions, insulin, adrenalin, 64.
Corynebacterium diphtheriae, reduction of potassium tellurite, 272
Cyanide, iodoacetate, survival, 553.

Dark adaptation, thyroid, α-dinitrophenol, 180.
Delvinal sodium, 233.
Diabetes, NaCl, glucose disposition, 287.
  insipidus, electrolytes, 682.
  hypophysis, 678.

Egg, fertilized, shearing force, 393.
  white disease, vit. B_6, fatty acids, 353.
Embryonic fluid, serology, influenza, 713.
  material, growth, 18.
Encephalitis, transmission, 601.
  virus, 609.
Encephalomyelitis, equine, rabies, 69.
Endocarditis lenta, heart sounds, 545.
Endocrine organs, *in vitro* maintenance, 184.
Enteritis, ulcerative, cause, 250.
Erythrocyte fragility, 640.

Fat metabolism, 417.

Gastric elimination of dyes, 295.
Gizzard erosions, bile acids, vit. K, cincophen, 168.
Glucose utilization, yeast, 443.
Gonads, ant. pituitary implants, 537.
Gonococci, non-pathogenic, 547.
Gramicidin, hemolytic effect, 182.
Gum acacia, non-available foodstuff, 178.

Heart electrogram, temperature, 395.
  lanatoside C, 651.
  lymph, function, 598.
  R. auricle catheterized, 462.
  sounds, infarction, 574.
Hemophilia, oxalic acid, 496.
Histamine, edema, 75.
Histiocytes, mobility, 635.
Hormone
  Androgen, assay, 1.
  Androsterone, pituitary, mamma, 265.
  Desoxycorticosterone, androgen, 336.
  α-Estradiol benzoate, azoospermia, 174.
  Estrogen, mating behavior, hypophysectomy, 669.
    tumoral specificity, 161.
    tumorigenic, 76, 164.
    uterine tumor, 262.
  Estrone inactivation by liver, 276.
  Insulin, glycogenesis, sugar conc, 390.
  Isoandrosterone from testosterone, 351.
    in virilism urine, 51.
  Lactogen, AP, litter size, 670.
    placentoma, 586.
  Progesterone anesthesia, 197.
    assay, 440.
    deciduomata, 698.
  Secretin, assay, 149.
  Sodium pregnandiol glucuroniadate, chimpanzee urine, 57.
  Steroid, anesthesia, 116.
  Testosterone, anal fin transformation, 283.
    anesthesia, 142.
    inactivation, 452.
  Thyroid, dark adaptation, 180.
Hydrofluoric acid burn, treatment, 190.
Hypertension, blood vol, 66.
  production, kaolin, 696.
"Hypotensive" extr, 220.

Influenza, repeated, 316.
  virus, 371.
  purification, 709.
Iron, radio, secretion in milk, 284.

Ketosis, high metabolism, 703.
Kidney conc. test, pituitary extr., 688.
  function, hypophysectomy, 44.
  pressor amines, 343.
  tubules, reabsorption amino acids, 129.

Lead poisoning, Na citrate treatment, 476.
Leishmania donovani, growth, 606.
Leucocytosis, production, 5.
Liver cirrhosis, Vit. B, 502.
  extr, embryo blood picture, 194
  Fe, 207.
  glycogen, tyrosine, 671.
  injury, dietary, 492.
Lymph, dye passage from blood, 549.
Lymphogranuloma venereum, antigen, 300.
Lymphoma implants, nuclei injection, 428.

Mamma, growth, involution, 437.
  growth, nutrition, estradiol, 379.
Melanin deposition, testosterone, 649.
Meningitis, influenza, therapy, 34.
Metakentrin, assay, 644.
Milk, soft curd, 593.
Morphine, codeine, synergism by sulfapyridine, 53.
Muscle extr., toxicology, 228.

Neisseria gonorrhoeae, capsulation, 700.
Nerve centers, 14.
  stimulation, electrical fields, 532.
Neuromuscular disease, α-tocopherol testosterone, creatine excretion, 94.
Neuronal synchronization, 627.
Nucleoprotein, tumor metabolic activity, 403.

Olfactory cells degeneration, 41.
Optic system activity, 557.
Ovary, hysterectomy, 580.

Papilloma virus protein, 419.
Pectin, intestinal decomposition, 569.
Pellagra—nicotinic acid excretion, 374.
Pentothal, thioethamyl, depressants, 98.
Perosis, choline, gelatin, creatine, 155.
Phage production, thermal inhibition, 210.
Pityrosporum ovale, characteristics, 176.
Pneumococci, sulfapyridine fast, 241.
Pneumonia, jaundice blood O_2, 554.
Polyarthritis, jaundice, 257.
Procaine convulsions, Hg, Pb, 309.
Propionic acid, bacterial synthesis, 313.
Prothrombin, 357.
  stability, 80.
  time, Na citrate, 136.
Pulmonary capacity detn, 266.
Pyruvate, brain oxidations, 121.

"Q" fever, complement fixation, 665.
Quinine hypersensitivity, 223.

Radio Ca, Sr, 86.
  metabolism, 91.
Radiosensitivity, new-born, low temp., 662.
Ragweed protein, 153.
Reflex, afferent stimulation, 543.
  copulatory, adrenal cortical extr, 239.
Relapsing fever spirochete growth, 638.
  therapy, 658.
Renin, B.P. reduction, 104.
  cardiac output, 200.
Reproduction, pro-oxidants, 399.
  soybean meal, 290.
Ribonuclease, 436.
Rickettsia vaccine, 223.

Scillaridin, theophyllinated, toxicity, 36.
Sclerema neonatorum, serum lipids, 325.
Semen, metabolic rate, 455.
Sexual behavior, thyroidectomy, 16.
Shock, epinephrine, 385.
  liver histamine, 576.
  prevention, histaminase, 187.
Skin, frog, electrical energy production, 382.
Sphingomyelin, radio P, 281.
Sr, radio, 86.
  metabolism, 91.
Stem "tracheal" fluid, minerals, 347.
Streptococcus fibrinolysin detn., 674.
  p-caproylaminobenzenesulfonhydroxamide, sulfanilamide, 364.
Sulfonamides, p-aminobenzoic acid, 59, 131, 133.
  avian malaria, 523.
  bile, 484.
  E. coli, 591.
  gas gangrene, 9.
  gonococci, 527.
  in tissues, 31.
  intraperitoneal, 458.
  plasma lipids, 83.
  pneumonia, 361.
  renal function, 124.
  tolerance, 79.

Taenia Taeniaformis infection, splenectomy, 29.
Tetrahymena geleii, nutrition, 482.
Thymectomy, pituitary growth hormone, 411.
Thyroid disease, BMR, respiration, 596.
Thyrotropic thyroid response, 306.
Tissues, calcified, composition, 62.
Tooth eruption, hibernation, 685.
Triacetin, toxicity, 26.
Trichomonas *foetus* and *vaginalis,* growth, 489.
Tuberculin antibodies, 47.
  assay, 615.
Tuberculosis, M. histiocytes, 630.
Tumor production, estrogen, 76, 161, 164.
  uterine, production by estrogen, 262.
Tyrosinase, glutathione, dopa reaction, 269.

Uremia, desoxycorticosterone, blood N P N, 541.
Urogastrone, pituitrin, 691.
Urolithiasis, urine citrate, 216.

Vaginal epithelium, hormone effects, colchicine, 330.
Vasopressin, diencephalon, 629.
Virus influenza, 371.
    purification, 709.
  papilloma, 419.
  pneumonitis in newborn, 114.
  poliomyelitis, 22, 319.
    adaptation, 431.
Vit. A, blood, 248.
  B_6, egg white disease, 353.
  Biotin, synthesis, 449.
  C. deficiency, anemia, 603.
  K absorption, bile fistula, 415.
  administration, 535.
  deficiency, 421.
  gizzard erosions, 168.
  nicotinic acid excretion, pellagra, 374.
  sulfapyridine antagonist, 369.
  pantothenic acid, achromotrichia, 507.
  growth, graying, 655.
  acid, inositol, alopecia, 565.
  riboflavin, tissue, 146.
  need, fat intake, 100.
  thiamin absorption, 387.
Vitamins needed by rat, 530.